MONOCLONAL ANTIBODIES AS A TOOL FOR EVALUATION OF THE PROTEIN CHANGES **DURING BULL SPERM CAPACITATION.**

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The process of fertilization is characterized by series of complex set of events. After ejaculation, sperms are able to move actively but lack fertilizing competence. They acquire ability to fertilize in a female genital tract in a time-dependent process called capacitation. Before a spermatozoon can fertilize an oocyte, it must undergo a cascade of biochemical and physiological changes that facilitates its binding and penetration into the oocyte. Capacitation involves reorganization of membrane proteins, an increase in membrane fluidity, cholesterol efflux, ion fluxes resulting in alteration of sperm membrane potential, increased tyrosine phosphorylation of proteins, induction of hyperactivation. The aim of our study were monoclonal antibodies used to detect changes of sperm surface proteins reaction patterns after capacitation.

Material and methods

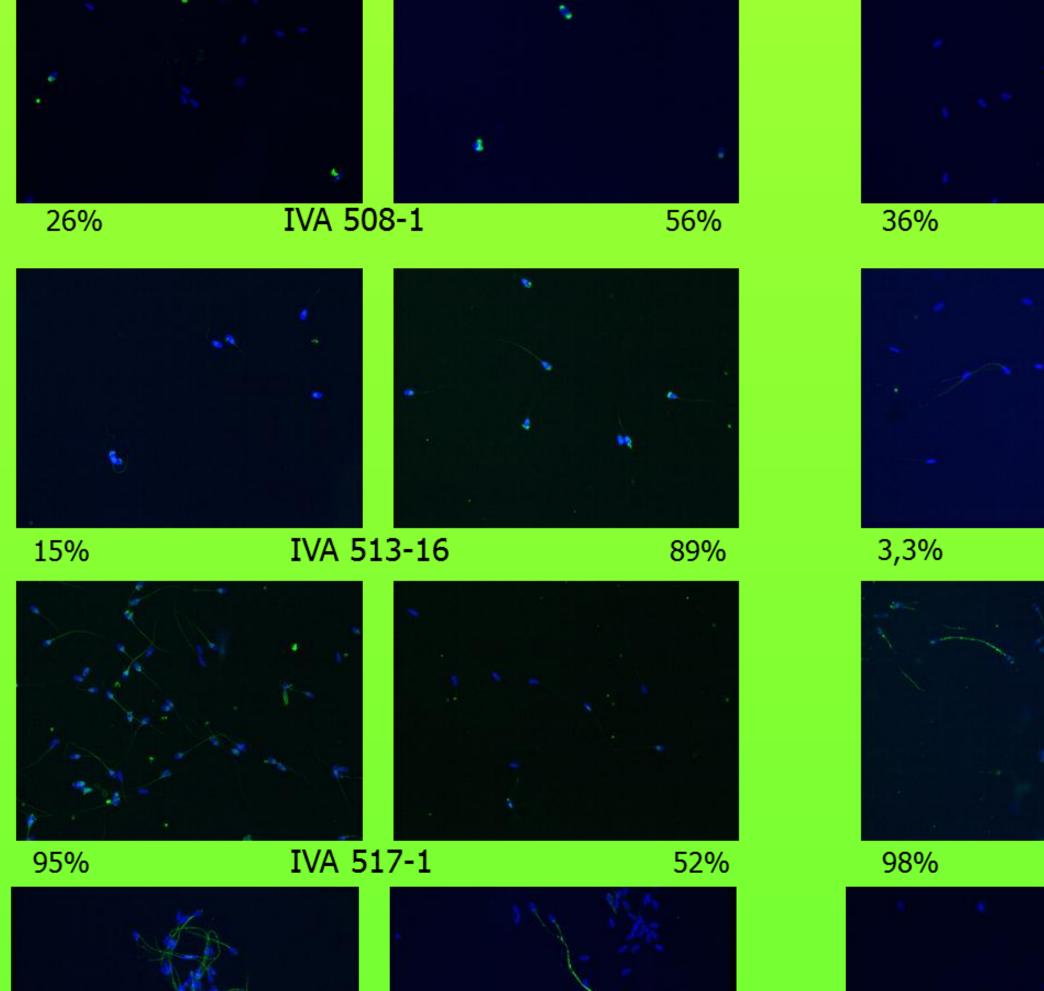
In our study a set of 34 anti-sperm monoclonal antibodies were used to detect changes of bovine sperm surface proteins reaction patterns after capacitation induced by TL Sperm cell capacitation medium (Minitube). Monoclonal antibodies (mAbs) were produced by hybridoma cell lines obtained after intrasplenic immunization of BALB/c mice with intact bull sperm. The changes in the reaction patterns were evaluated by indirect immunofluorescence, PAGE-SDS and two-dimensional gel electrophoresis followed by western blot analysis with anti-sperm mAbs and anti-phosphotyrosine α -PY antibody

Results

MAb	Capacitated sperm	Non-capacitated	Capacitated sperm	Non-capacitated sperm	Capacitated sperm	Non-capacitated sperm	Capacitated sperm	Non-capacitated sperm
IVA 502-3	64,4 %, acrosome, tail	70 %, acrosome, tail						
IVA 502-33	97 %, acrosome, tail	75 %, acrosome, tail						
IVA 508-1	26 %, acrosome	56 %, acrosome						

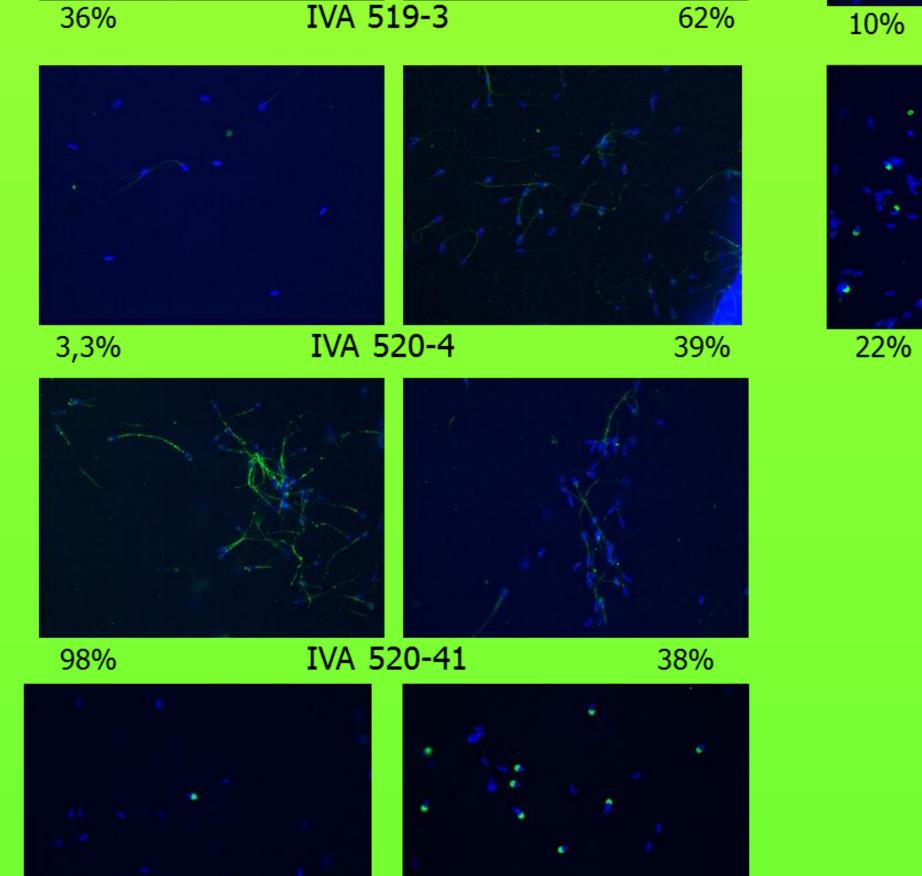
INDIRECT IMMUNOFLUORESCENCE

NA 508-3 52 %, acrosome 56 %, acrosome NA 510-4 89 %, head, tail 90 %, head, tail NA 513-1 - - VA 513-15 51 %, acrosome, postacr.region, tail 40 %, acrosome, postacr.region, tail NA 513-16 15 %, acrosome, postacr.region, tail 89 %, acrosome, postacr.region, tail NA 513-2 64 %, acrosome, postacr.region, tail 72 %, acrosome, postacr.region, tail NA 513-8 40 %, head 40 %, head 10 %, head NA 513-8 40 %, head 40 %, head 10 %, acrosome, postacr.region, tail NA 513-8 40 %, head 40 %, head 10 %, head NA 513-8 90 %, corosome, postacr.region, tail 72 %, acrosome, postacr.region, tail NA 513-8 90 %, whole sperm 90 %, whole sperm 90 %, whole sperm NA 518-1 90 %, whole sperm 90 %, whole sperm NA 518-1 90 %, acrosome NA 519-3 36.2 %, acrosome 62 %, acrosome 87.6 %, 90 %, tail 10 % 520-41 NA 520-41 98 %, tail 37.5 %, tail 33.3 %, acrosome, postacr.region, tail NA 526-7 10.1 %	IVA 508-1	26 %, acrosome	56 %, acrosome
IVA 513-1 - - IVA 513-15 51 %, acrosome, postacr.region, tail 40 %, acrosome, postacr.region, tail IVA 513-16 15 %, acrosome, postacr.region, tail 89 %, acrosome, postacr.region, tail IVA 513-2 64 %, acrosome, postacr.region, tail 72 %, acrosome, postacr.region, tail IVA 513-3 40 %, head 40 %, head 40 %, head IVA 514-4 50 %, postacr.region, tail 72 %, acrosome, postacr.region, tail IVA 517-1 95 %, postacr.region, tail 52 %, postacr.region, tail IVA 518-1 90 %, whole sperm 90 %, whole sperm VA 518-1 90 %, ushole sperm 90 %, whole sperm IVA 519-2 32,8 %, acrosome 48,8 %, acrosome IVA 520-4 3,3 %, tail 39 %, tail IVA 520-41 98 %, tail 37,5 %, tail IVA 520-42 98 %, tail 18,8 %, tail IVA 526-7 10,1 %, acrosome, postacr.region, tail 42,9 %, acrosome, postacr.region, tail IVA 526-7 36,8 %, acrosome, postacr.region, tail 42,9 %, acrosome, postacr.region, tail IVA 527-1 31,3 %, acrosome, postacr.region, tail 42,9 %, acrosome, postacr.region, tail IVA 527-1 <td< td=""><td>IVA 508-3</td><td>52 %, acrosome</td><td>56 %, acrosome</td></td<>	IVA 508-3	52 %, acrosome	56 %, acrosome
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IVA 520-41 98 %, tail 37,5 %, tail IVA 520-42 98 %, tail 18,8 %, tail IVA 520-42 98 %, tail 18,8 %, tail IVA 526-7 10,1 %, acrosome, postacr.region, tail 33,3 %, acrosome, postacr.region, tail IVA 526-9 36,8 %, acrosome, postacr.region, tail 42,9 %, acrosome, postacr.region, tail IVA 527-1 31,3 %, acrosome, postacr.region, tail 37,5 %, acrosome, postacr.region, tail IVA 527-5 85,5 %, head 72,1 %, head IVA 527-10 9,63 %, acrosome 40,7 %, acrosome IVA 527-10 9,63 %, acrosome 40,7 %, acrosome IVA 534-4 86,1 %, head 78 %, head IVA 534-7 79 %, head 51,4 %, head IVA 537-2 76,1 %, tail 84 %, tail IVA 537-3 80,4 %, tail 85,3 %, tail IVA 537-5 74,4 %, tail 60 %, tail IVA 543-21 96,9 %, whole sperm 91,8 %, whole sperm IVA 543-21 99,3 %, whole sperm 99,3 %, whole sperm	IVA 519-3	36,2 %, acrosome	62 %, acrosome
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WA 526-7 10,1 %, acrosome, postacr.region, tail 33,3 %, acrosome, postacr.region, tail WA 526-9 36,8 %, acrosome, postacr.region, tail 42,9 %, acrosome, postacr.region, tail WA 527-1 31,3 %, acrosome, postacr.region, tail 37,5 %, acrosome, postacr.region, tail WA 527-5 85,5 %, head 72,1 %, head WA 527-10 9,63 %, acrosome 40,7 %, acrosome WA 534-4 86,1 %, head 78 %, head WA 534-7 79 %, head 51,4 %, head WA 537-2 76,1 %, tail 84 %, tail WA 537-3 80,4 %, tail 85,3 %, tail WA 537-5 74,4 %, tail 60 %, tail WA 543-21 96,9 %, whole sperm 91,8 %, whole sperm WA 543-21 90,3 %, whole sperm 99,3 %, whole sperm	IVA 520-41	98 %, tail	37,5 %, tail
IVA 526-9 36,8 %, acrosome, postacr.region, tail 42,9 %, acrosome, postacr.region, tail IVA 527-1 31,3 %, acrosome, postacr.region, tail 37,5 %, acrosome, postacr.region, tail IVA 527-5 85,5 %, head 72,1 %, head IVA 527-10 9,63 %, acrosome 40,7 %, acrosome IVA 527-10 9,63 %, acrosome 40,7 %, acrosome IVA 527-10 9,63 %, acrosome 40,7 %, acrosome IVA 534-4 86,1 %, head 78 %, head IVA 534-7 79 %, head 51,4 %, head IVA 537-2 76,1 %, tail 84 %, tail IVA 537-3 80,4 %, tail 85,3 %, tail IVA 537-5 74,4 %, tail 60 %, tail IVA 543-21 96,9 %, whole sperm 91,8 %, whole sperm IVA 543-21 99,3 %, whole sperm 99,3 %, whole sperm	IVA 520-42	98 %, tail	18,8 %, tail
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Image: Non-State State	IVA 526-9	36,8 %, acrosome, postacr.region, tail	42,9 %, acrosome, postacr.region, tail
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IVA 534-4 86,1 %, head 78 %, head IVA 534-7 79 %, head 51,4 %, head IVA 537-2 76,1 %, tail 84 %, tail IVA 537-3 80,4 %, tail 85,3 %, tail IVA 537-5 74,4 %, tail 60 %, tail IVA 543-21 96,9 %, whole sperm 91,8 %, whole sperm IVA 543-2 91,9 %, whole sperm 99,3 %, whole sperm IVA 543-21 99,3 %, whole sperm 99 %, whole sperm	IVA 527-5	85,5 %, head	72,1 %, head
IVA 534-7 79 %, head 51,4 %, head IVA 537-2 76,1 %, tail 84 %, tail IVA 537-3 80,4 %, tail 85,3 %, tail IVA 537-5 74,4 %, tail 60 %, tail IVA 543-21 96,9 %, whole sperm 91,8 %, whole sperm IVA 543-2 91,9 %, whole sperm 99,3 %, whole sperm IVA 543-21 99,3 %, whole sperm 99 %, whole sperm	IVA 527-10	9,63 %, acrosome	40,7 %, acrosome
IVA 537-276,1 %, tail84 %, tailIVA 537-380,4 %, tail85,3 %, tailIVA 537-574,4 %, tail60 %, tailIVA 543-2196,9 %, whole sperm91,8 %, whole spermIVA 543-291,9 %, whole sperm99,3 %, whole spermIVA 543-2199,3 %, whole sperm99 %, whole sperm	IVA 534-4	86,1 %, head	78 %, head
IVA 537-3 80,4 %, tail 85,3 %, tail IVA 537-5 74,4 %, tail 60 %, tail IVA 543-21 96,9 %, whole sperm 91,8 %, whole sperm IVA 543-2 91,9 %, whole sperm 99,3 %, whole sperm IVA 543-21 99,3 %, whole sperm 99 %, whole sperm	IVA 534-7	79 %, head	51,4 %, head
IVA 537-5 74,4 %, tail 60 %, tail IVA 543-21 96,9 %, whole sperm 91,8 %, whole sperm IVA 543-2 91,9 %, whole sperm 99,3 %, whole sperm IVA 543-21 99,3 %, whole sperm 99 %, whole sperm	IVA 537-2	76,1 %, tail	84 %, tail
IVA 543-21 96,9 %, whole sperm 91,8 %, whole sperm IVA 543-2 91,9 %, whole sperm 99,3 %, whole sperm IVA 543-21 99,3 %, whole sperm 99 %, whole sperm	IVA 537-3	80,4 %, tail	85,3 %, tail
IVA 543-2 91,9 %, whole sperm 99,3 %, whole sperm IVA 543-21 99,3 %, whole sperm 99 %, whole sperm	IVA 537-5	74,4 %, tail	60 %, tail
IVA 543-21 99,3 %, whole sperm 99 %, whole sperm	IVA 543-21	96,9 %, whole sperm	91,8 %, whole sperm
	IVA 543-2	91,9 %, whole sperm	99,3 %, whole sperm
IVA 582 21,5 %, acrosome 47,7 %, acrosome	IVA 543-21	99,3 %, whole sperm	99 %, whole sperm
	IVA 582	21,5 %, acrosome	47,7 %, acrosome

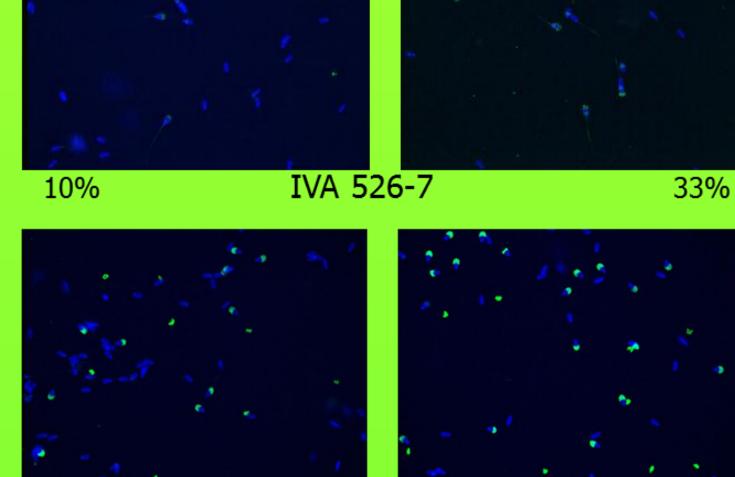


19%

10%



IVA 527-10



IVA 582

48%

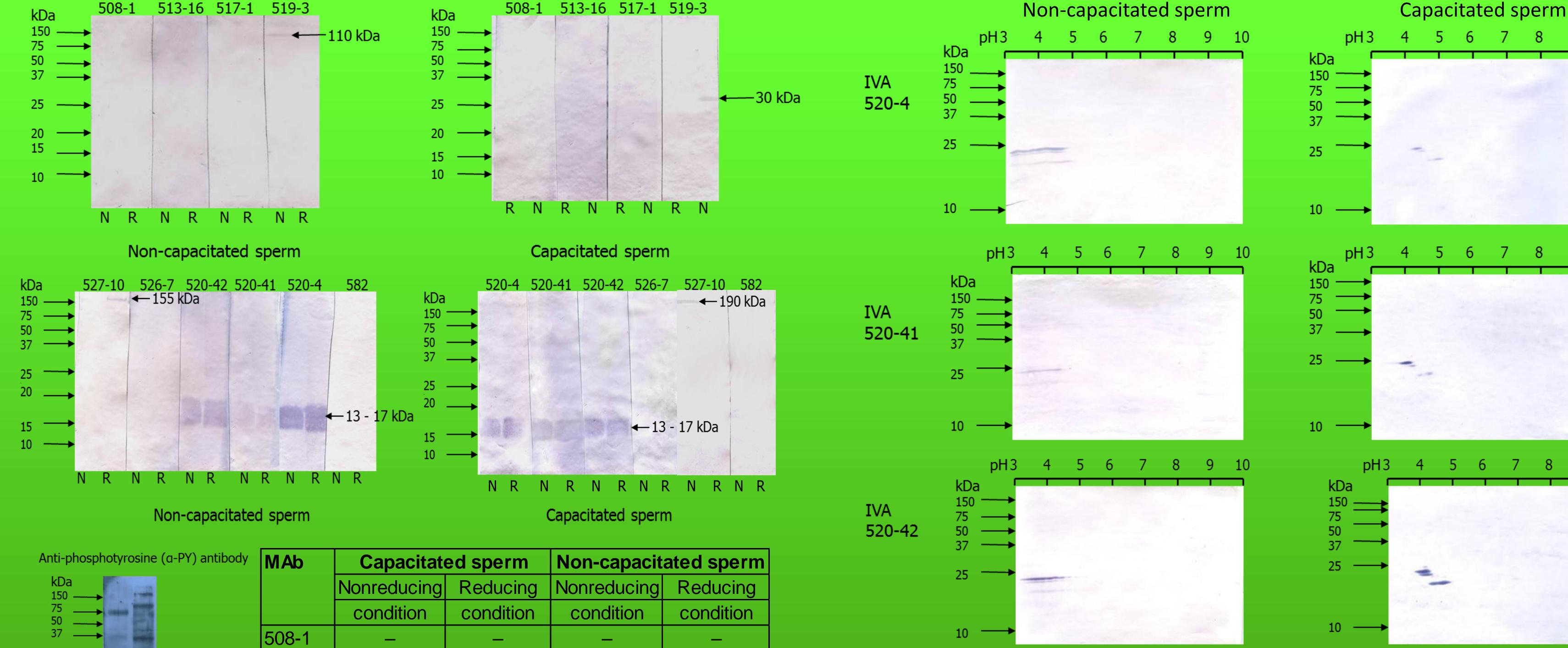
10

TWO-DIMENSIONAL GEL ELECTROPHORESIS

Non-capacitated sperm							Ca	apac	itat	ed s	ре		
nH3	4	5	6	7	R	٩	10	nH 3	4	5	6	7	1

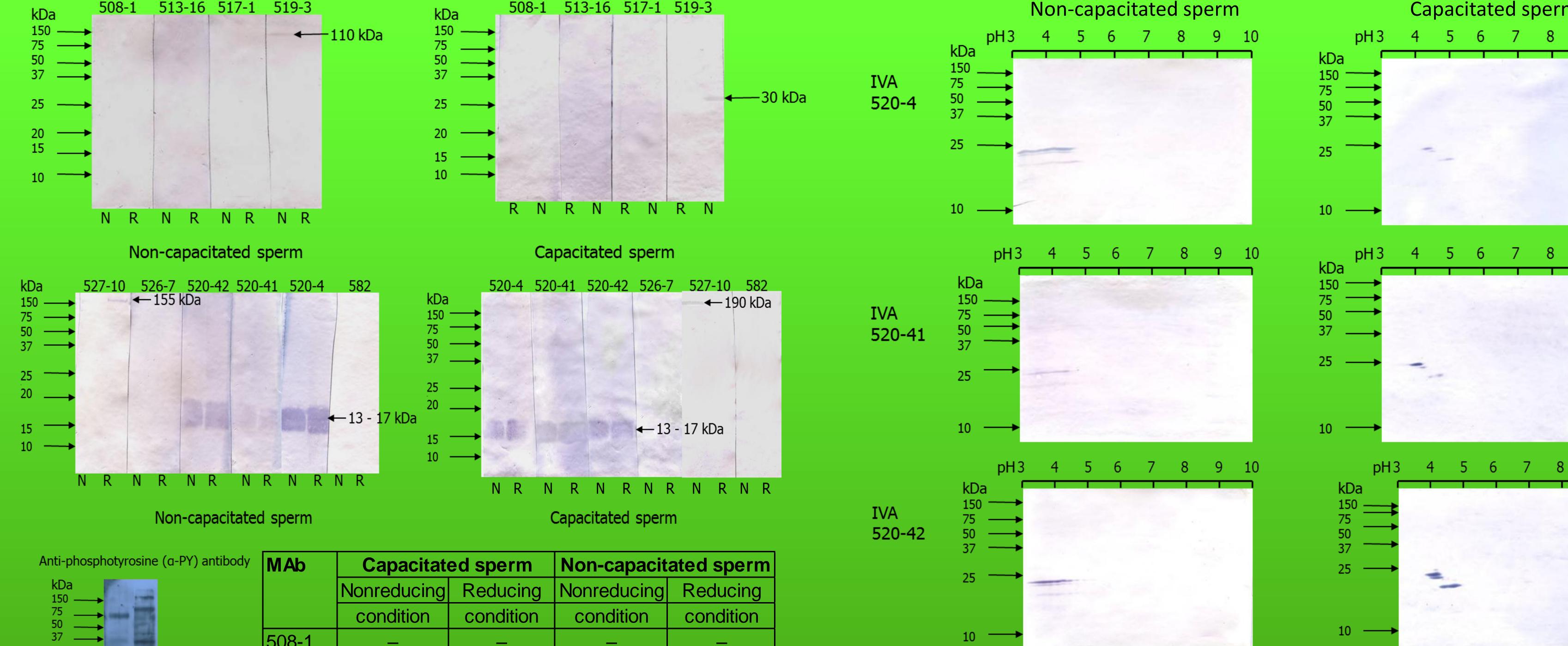
41%





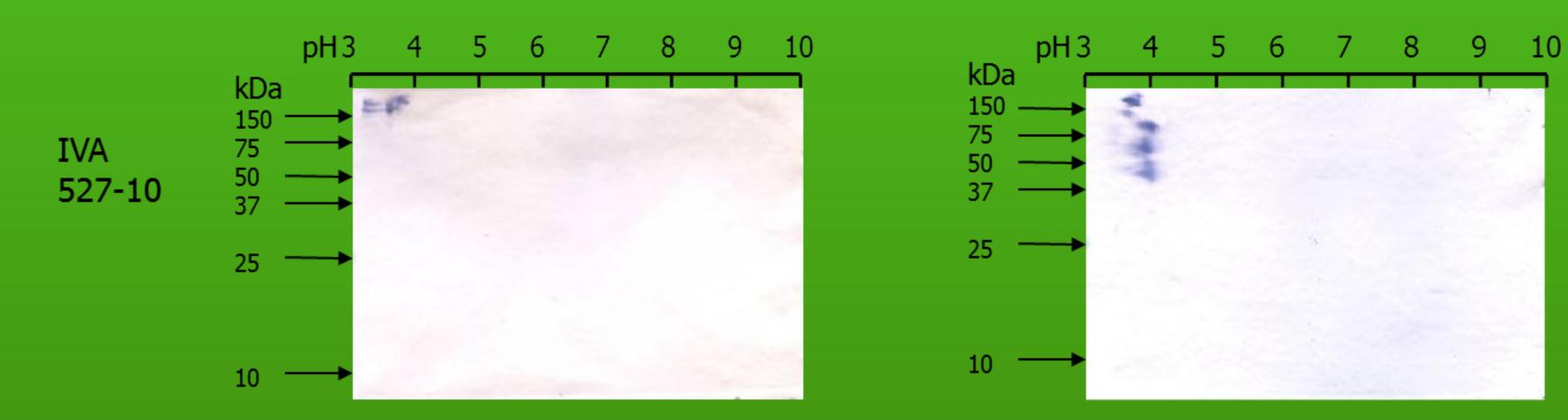
IVA 520-42

98%



25	\rightarrow			
25 20	\rightarrow		111	
15 10	\rightarrow			
10	>	4.00	1.20	
		Capacitated sperm	Ion-capacitated sperm	

513-16	—	—	—	—
517-1		_		
519-3	30 kDa	_	110 kDa	_
526-7		_	_	_
527-10		190 kDa		155 kDa
520-4	14 – 16 kDa	14 – 16 kDa	13 – 17 kDa	13 – 17 kDa
520-41	14 – 16 kDa	14 – 16 kDa	13 – 17 kDa	13 – 16 kDa
520-42	14 – 16 kDa	14 – 16 kDa	13 – 18 kDa	12 – 17 kDa
543-2				



Conclusion

- The changes were observed in indirect immunofluorescence in the percentage of reactive sperm
 - The percentage of reactive sperm after treating with mAbs IVA 508-1, IVA 513-16, IVA 519-19, IVA 520-4, IVA 526-7, IVA 527-10 and IVA 582 increase approximately twice
 - The percentage of reactive sperm after treating with mAbs IVA 517-1, IVA 520-41 and IVA 520-42 decrease approximately twice
- Western blot analysis shown changes in molecular weight of proteins detected by mAbs IVA 519-3, IVA 520-4, IVA 520-41, IVA 520-42 and IVA 527-10
- Two-dimensional PAGE and western blot analysis of proteins detected by mAbs IVA 520-41, IVA 520-42 and IVA 527-10 shown that reactive region changed to less acidic region after capacitation
- For statistical evaluation all experiments should be repeated

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