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

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Introduction

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.

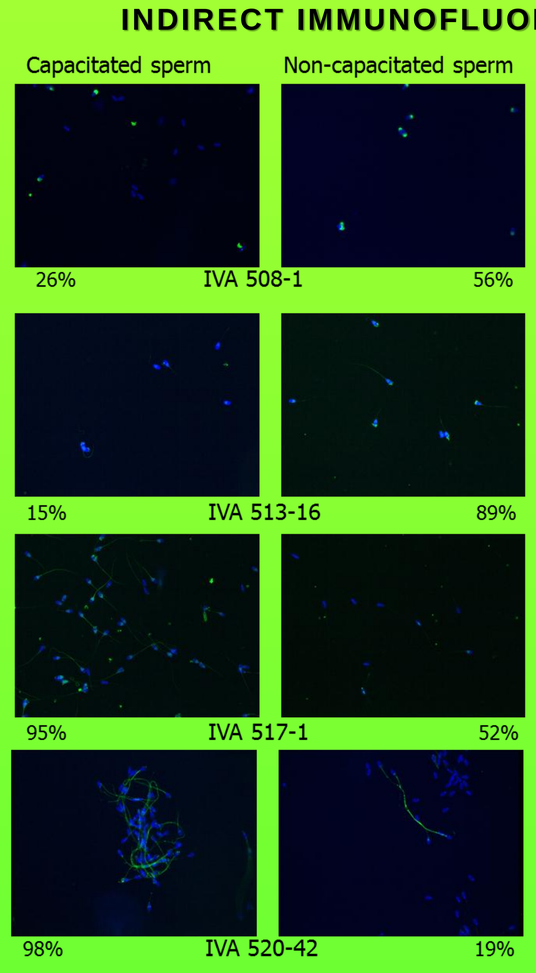
Material and methods

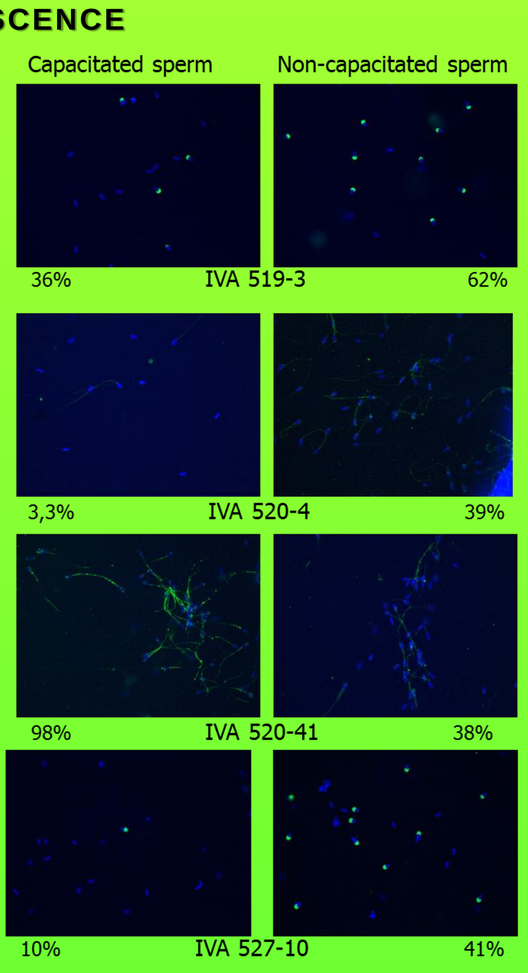
In our study a set of 34 anti-sperm monoclonal antibodies were used to detect changes of bovine sperm surface proteins reaction patterns after capacitation induced by TL Sperm cell capacitation medium (Minitube). Monoclonal antibodies (mAbs) were produced by hybridoma cell lines obtained after intrasplenic 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

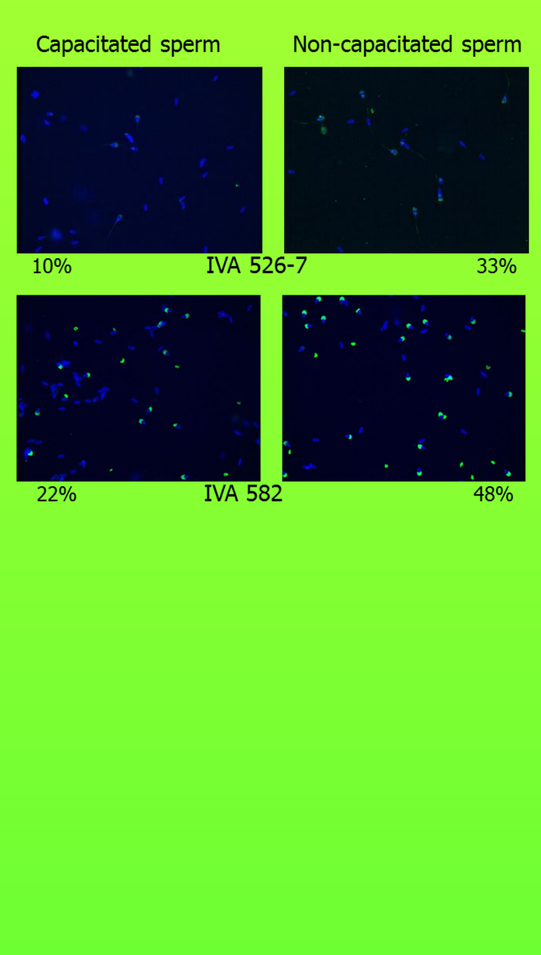
Results

INDIRECT IMMUNOFLUORESCENCE

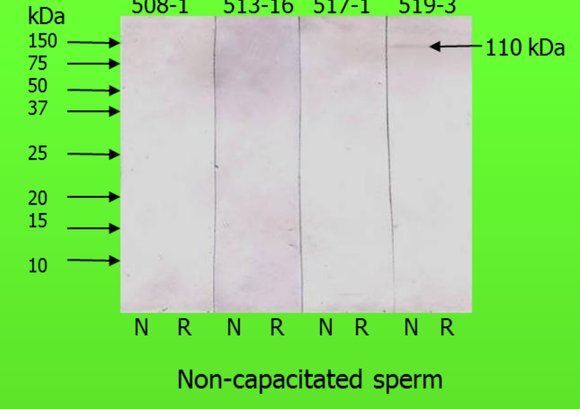
MAb	Capacitated sperm	Non-capacitated
IVA 502-3	64,4 %, acrosome, tail	70 %, acrosome, tail
IVA 502-33	97 %, acrosome, tail	75 %, acrosome, tail
IVA 508-1	26 %, acrosome	56 %, acrosome
IVA 508-3	52 %, acrosome	56 %, acrosome
IVA 510-4	89 %, head, tail	90 %, head, tail
IVA 513-1	-	-
IVA 513-15	51 %, acrosome, 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 513-8	40 %, head	40 %, head
IVA 514-4	50 %, acrosome, postacr.region, tail	72 %, acrosome, postacr.region, tail
IVA 517-1	95 %, postacr.region, tail	52 %, postacr.region, tail
IVA 517-3	53 %, postacr.region, tail	46,6 %, postacr.region, tail
IVA 518-1	90 %, whole sperm	90 %, whole sperm
IVA 518-11	97,7 %, 90 %, celá spermia	87,6 %, 90 %, whole sperm
IVA 519-2	32,8 %, acrosome	48,8 %, acrosome
IVA 519-3	36,2 %, acrosome	62 %, 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	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 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 582	21,5 %, acrosome	47,7 %, acrosome

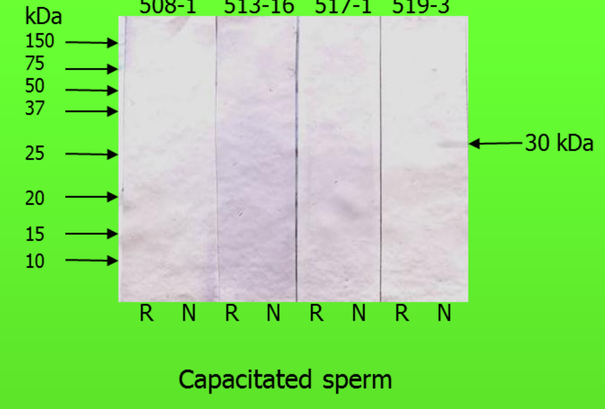


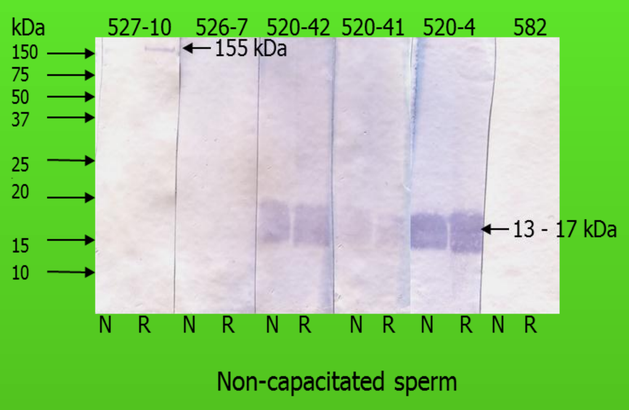


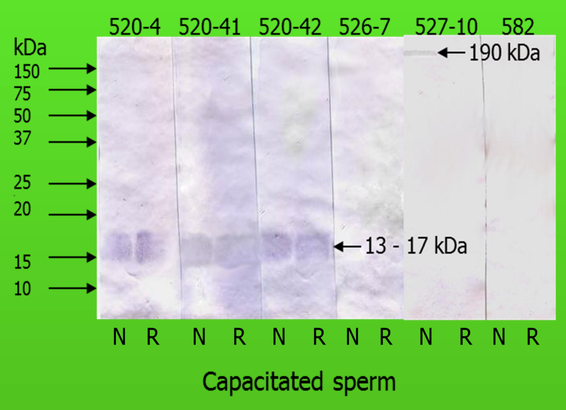


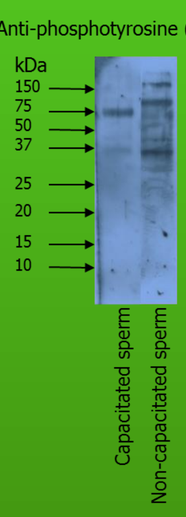
WESTERN BLOT ANALYSIS





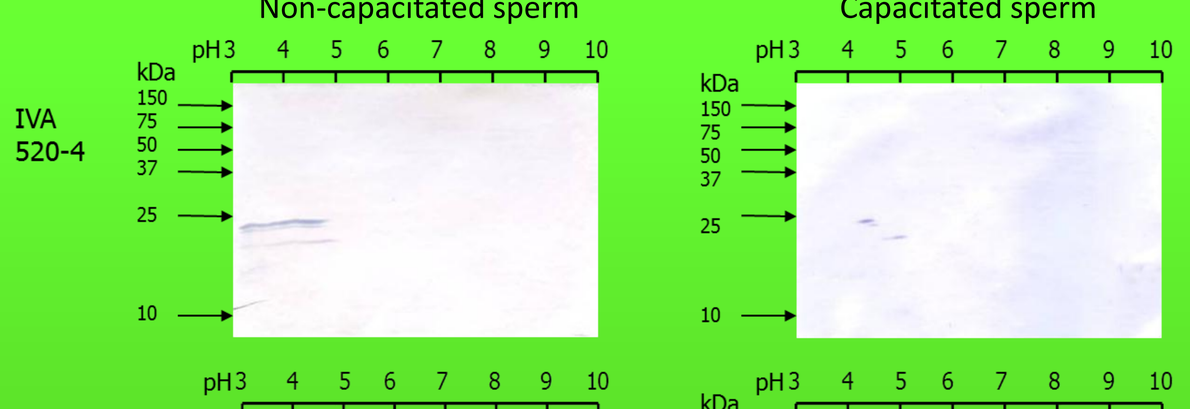






MAb	Capacitated sperm		Non-capacitated sperm	
	Nonreducing condition	Reducing condition	Nonreducing condition	Reducing condition
508-1	–	–	–	–
513-16	–	–	–	–
517-1	–	–	–	–
519-3	30 kDa	–	110 kDa	–
526-7	–	–	–	–
527-10	–	190 kDa	–	155 kDa
520-4	14 – 16 kDa	14 – 16 kDa	13 – 17 kDa	13 – 17 kDa
520-41	14 – 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	–	–	–	–

TWO-DIMENSIONAL GEL ELECTROPHORESIS



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 520-4, 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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