

STATUS OF NATURALLY DEVELOPING ANTI SPERM ANTIBODIES IN SERUM OF CALVES, HEIFERS, COWS AND THEIR EFFECT ON *IN VITRO* CAPACITATION AND ACROSOME REACTIONAnkit K Ahuja¹, Ranjna S Cheema* and Ajeet Kumar²¹MVSc student, * Senior Physiologist (Reprod), ²Assist Professor(Received on Date: 12th September 2016)Date of Acceptance: 15th November 2016)**ABSTRACT**

The study was planned with an objective to assess the status of naturally developing antisperm antibodies (ASA) in the blood serum of cross bred (HF x Red Dane x Sahiwal) calves, heifers and cows by Sperm Mar test and ELISA. Number of IgG/IgA latex particles attached to the head, tail or both of spermatozoa in the presence of serum of calves, heifers and cows were 1 - 3, 1 - 5 and > 5. A significant ($P < 0.05$) decrease in antibody positivity was observed in blood serum of calves from 7 to 365 days of age. A significant ($P < 0.05$) decrease in spermatozoa bound to IgG and IgA latex particles and percent positivity for ASA was observed in the heifers and cows exhibiting variable number of AIs from 1st / 1st post-partum estrous to pre-conception. This study revealed an increase in percentage of calves with > 40% or more reaction between the coated latex particles and motile spermatozoa with advancing age and a decrease in percentage of heifers and cows from 1st / 1st post-partum estrous to pre-conception. Supplementation of 1:50 diluted blood serum of calves, heifers and repeat breeder cows to TALP significantly ($P < 0.05$) declined the percentage of capacitated, viable spermatozoa. But an increase in acrosome reacted spermatozoa was observed in the presence of serum of heifers and repeat breeder cows as compared to control, which was significant ($P < 0.05$) only in the presence of serum of heifers. The study concluded that naturally developing antibodies in blood serum of calves, heifers and cows are reactive to spermatozoa and delays the fertility in heifers and cows.

Keywords: Developing, Antisperm antibodies, Cross bred calves, heifers, Cows.

No : of Tables:3**No: of Figures : 3****No: of References : 32**

INTRODUCTION

Anti-sperm antibodies (ASAs) are antibodies developed against the spermatozoa and can affect the sperm's fertility, thus causing sub fertility / infertility / repeat breeding in humans and animals. ASAs can be present in the blood serum, cervical mucus, oviductal fluid, uterine fluid, follicular fluid or seminal plasma of females. Presence of ASAs can inhibit passage of spermatozoa through cervical mucus, prevent membrane fluidity changes needed for capacitation, reduce the ability of spermatozoa to undergo the acrosome reaction and interfere with binding to the zona pellucida and fertilization (Fijak and Meinhardt, 2006). Immunological incompatibility of the sperm and oocyte because of production of ASAs has been documented (Tas *et al.*, 2007) in both cattle and buffaloes. Since spermatozoa have to come in contact with blood to develop ASAs in the animal body; therefore inflammatory states, such as metritis and vaginitis, or trauma and bleeding occurring during mating have an important role in the development of these antibodies (Panchal *et al.*, 1990, Risvanli *et al.*, 2003). During passage through male and female genital tracts, spermatozoa are surrounded by seminal plasma that contains strong immunosuppressive molecules which block recognition and antigen processing (James and Hargreave, 1984). Other molecules mask antigens important for the fertilization process on the surface of spermatozoa. In addition, cervical mucus, uterine and follicular fluid also contains immunosuppressive substances (Landers *et*

al., 1994). One of the basic characteristics of sperm cells is a continuous change of their antigenic structure due to the loss of surface molecules during maturation and following insemination. In the technology of artificial insemination (AI), the antigenic structure of sperm cells is changed due to the addition of different extenders, freezing and thawing procedures and reduction of seminal plasma volume. Because of the mentioned reasons, ASA may develop in the reproductive tract of female and affect the fertility. Prevalence of ASA has been studied in cattle using immuno peroxide assay (Fayemi, 2005), immunofluorescence (Milovanovic *et al.*, 2005), ELISA (Zrally *et al.*, 2003; Risvanli *et al.*, 2003 and Sarma *et al.*, 2009). Occurrence of ASA in blood serum or CM of cows has been related to age (Waziri and Fayemi, 2000; Fayemi *et al.*, 2005), parity (Zrally *et al.*, 2003; Waziri and Fayemi, 2000), repeat breeding/ number of inseminations and infertility (Risvanli *et al.*, 2002; Zrally *et al.*, 2003 and Sarma *et al.*, 2009, Cheema and Bansal, 2015; Jarora *et al.*, 2016). Present study focuses on changes in status of naturally developing ASA in blood serum of calves with advancing age and heifers/cows with number of inseminations from 1st / 1st post partum estrus to pre-conception. An attempt was also made to observe the effect of blood serum of calves, heifers and cows on *in vitro* capacitation and acrosome reaction.

Material and Methods

Blood

All procedures for blood collection were performed in accordance with the guidelines approved by the Institutional Animal Ethical Committee, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India. Blood samples of 10 new born female calves, 20 heifers and 20 cows were collected from dairy farm, GADVASU, Ludhiana. Sampling was done from day 7 to 365 days at an interval of 45 days (calves) and at the onset of estrus till pre-conception (cows/heifers). Serum was harvested from the blood and inactivated by incubating at 56°C for 30 min.

Procurement of semen

Chilled semen of cow bulls was procured from semen freezing lab, dairy farm, GADVASU for analysis of ASA in blood serum of calves, heifers and cows by Sperm Mar test and ELISA.

Chemicals and Reagents

All AR grade chemicals of Sisco Research Laboratories, Sigma and BR Biochem were used for this study. Distilled water (DW) from Milliopre purification system (RO/Synergy) was used for the preparation of reagents.

Indirect SpermMar test with SpermMar kit (FertiPro, 2011)

Diluted inactivated serum 1/4 with Tyrode albumin lactate pyruvate (TALP) medium (92.9 mM NaCl, 4 mM KCl, 25.9 mM NaHCO₃, Na₂HPO₄, 10 mM CaCl₂·2H₂O, 0.5 mM MgCl₂·6H₂O, 1.3 mM sodium pyruvate, 7.6 mM sodium lactate and 20 mM HEPES), pH 7.4 and incubated at 37° C for 30 min. Chilled semen was washed

twice with TALP by centrifugation at 1000g for 3 min and sperm pellet was suspended in TALP to get a sperm concentration to 20 X 10⁶. Incubated 100 µl of the sperm suspension of motile spermatozoa with 100 µl of inactivated 1/4 diluted serum or cervical mucus for 1 hr at 37° C. Added 2 ml of TALP, mixed well and centrifuged for 10 min at 1500 rpm. Pellet was re-suspended with 50 µl of TALP. On a slide, mixed 10 µl of sperm suspension and 5 µl of Sperm Mar latex particles IgG / IgA, mixed, covered with cover slip, kept in humid chamber for 5 min and observed under bright field microscope at 400 X. Attachment of latex particles to the head / tail or whole sperm was observed. About 150 sperms in different fields were counted and percentage was calculated.

$$\% \text{ total binding} = \frac{\text{Number of sperms with bound latex particle (s)}}{\text{Total number of sperms counted}} \times 100$$

Extraction of sperm proteins (Cheema et al., 2011)

Sperm membrane proteins were extracted with sodium dodecyl sulphate (SDS). Chilled semen was washed twice with phosphate buffered saline (PBS), pH 7.4 by centrifugation at 3000 rpm for 5 min to get a dilutor and seminal plasma free sperm pellet. A pellet containing about 500 x 10⁶ spermatozoa was suspended in 1.0 ml of 2 % SDS in 62.5 mM Tris-HCl (pH 6.8) containing protease inhibitors (Cocktail, SERVA). Sperm suspension was sonicated at 20 Watts for 3 x 20 secs,

centrifuged at 10000 rpm for 15 min. Pellet was discarded and supernatant was concentrated through 3 kDa protein concentrators (Millipore) and total protein content was estimated by the method of Lowry *et al.*, (1951)

Detection of ASA in serum by Enzyme linked immune sorbant assay (ELISA)

For percent positivity of ASA in serum of calves, heifers and cows, indirect ELISA was performed (Crowther, 1995). Plates were pre-coated with 100 µl Poly D Lysine hydrobromide (100 µg/ml) for one hr at 37 °C and washed twice with PBS-T. Then incubated with 5 µg protein (sperm antigen) per well incubating at 37°C for three hrs. Washed thrice with PBS and incubated with 300 µl of 2 % BSA per well for overnight at 4°C to block antigen coating. Again washed thrice with PBS pH 7.4 and added 1:100 diluted blood serum into the wells and incubated at 37°C for three hrs. Washed again with PBS and incubated with 100 µl/well of HRP conjugated anti bovine IgG for three hrs at 37°C. Washed the plate twice with PBS and incubated with 100 µl of o-phenyldiamine + 0.06 % H₂O₂ as a substrate for 20 min at room temperature. Stopped the reaction with 5 N H₂SO₄ and measured the absorbance at 492 nm using ELISA reader (Tecan). Percent positivity was calculated by the formula:

$$\frac{\text{Absorbance of serum} - \text{Absorbance of negative control}^*}{\text{Absorbance of negative control}^*} \times 100$$

*In negative control, carbonate buffer without antigen and PBS without primary antibody were added.

In Vitro Capacitation and Acrosome Reaction

In vitro capacitation of cattle bull spermatozoa was performed to observe the effect of serum of calves, heifers and repeat breeder cows containing naturally developing ASA. One ml of chilled semen was taken in 15 ml graduated tube and washed twice with the basic TALP medium by centrifugation at 1000 rpm for 5 minutes. The sperm suspension was then re-suspended in the energy rich TALP (supplemented with 0.25 % glucose, 0.6 % bovine serum albumin (BSA), 10 mg of streptomycin and 100 µl of 0.1 % stock solution of heparin) and motile spermatozoa were separated by swim up and transferred to a micro-centrifuge tube. Motile sperm suspension was divided into four parts (125 x 10⁶ spermatozoa / ml). Part one was kept as control and parts 2, 3 and 4 were supplemented with serum to get a ratio of 1:50 (Treated). All samples were incubated at 37°C for 6 hrs. Capacitation / acrosome reaction was assessed by counting 200 spermatozoa in chlorotetracycline stained sperms under fluorescence microscope at 400 X (Olympus). Green fluorescence on acrosome, a band between acrosome and equatorial segment and on post-acrosomal cap was observed in uncapacitated, capacitated and acrosome reacted-spermatozoa, respectively (Fig 3). Capacitated / acrosome reacted spermatozoa were also analyzed for viability and lipid peroxidation (malondialdehyde, MDA generated / 10⁹ spermatozoa) by eosin – nigrosin staining and Buege and Steven, (1978) methods.

Statistical Analysis

Indirect Sperm Mar Test values for the Calf, Heifer and Cows were compared using Tukey-Kramer test. The antibody positivity by ELISA test was compared using Duncan multiple range test.

Results and Discussion

Developing antiperm / cross-reactive antibodies in blood serum of calves, heifers and cows

Percentage of spermatozoa bound to IgG/IgA latex particle in the presence of serum and percent positivity for ASA in serum of calves, heifers and cows showed a variation among the animals (Tables 1, 2).

3.1.1 Calves

Number of IgG/IgA latex particles attached to the head, tail or both of spermatozoa in the presence of serum of calves was only 1 - 3 (Fig 1a). Percentage of spermatozoa bound to IgG and IgA latex particles in the presence of serum of calves at the age of 7 and 365 days did not indicate any significant ($P > 0.05$) change in the level of IgG / IgA in the serum of calves with advancing age (Table 1). Percent positivity for ASA in serum of calves showed an increase from 7 to 135 days and thereafter a gradual decrease till 365 days. A significant ($P < 0.05$) total decrease of 23.66 % in antibody positivity was observed from 7- 365 days of age (Table 2). Lazarvic *et al.* (2002) revealed an increase in the ASA titre with the age of calf. Jimanez *et al.* (1986) and Lazarvic *et al.* (2002) did not find antibodies in sera of calves collected before colostrum ingestion. 1st positive reaction in samples tested for antisperm IgG was obtained 48

hrs after colostrum ingestion (Jimanez *et al.* 1986). It has been hypothesized that in calves ASA naturally occurring before puberty are most probably due to cross reactivity with microbial antigens. New born depends on antibody from colostrums (Silper *et al.*, 2012), which are derived from the serum rather than mammary glands (Porter, 1979). Therefore, detection of IgG /IgA type ASA and percent ASA positivity in serum of calves may be due to three reasons: 1) Immunoglobins derived from the colostrums; 2) cross reactive antibodies developed in the blood of calves in response to some microbial infection; 3) ASA or cross reactive antibodies of the mother that were derived from the blood to colostrums and then absorbed by the calves after colostrums ingestion.

Heifers

Number of IgG / IgA latex particles attached to the head, tail or both of spermatozoa in the presence of serum of heifers was 2 - 5 (Fig 1b). A significant ($P < 0.05$) decrease of 12.92 / 13.82 % and 7.97 / 15.25 % in spermatozoa bound to IgG and IgA latex particles was observed in the heifers exhibiting one AI / 2 - 3 AIs from 1st to pre-conception estrous (Table 1). A significant ($P < 0.05$) decline of 5.2 % and 3.8 % was observed in percent positivity for ASA in serum of heifers receiving one AI and 2 - 3 AIs from 1st estrus to pre-conception (Table 2). Risvanli *et al.* (2003) did not observe ASA in non-pregnant heifers but prevalence of ASA in serum of pregnant heifers was 18.18 %.

As heifers were not exposed to semen at the time of 1st estrous, therefore, presence of IgG / IgA type ASA and

positivity of serum for ASA may be only due to cross-reactivity of antigens with the same antibody. It may not be because of antibodies absorbed from colostrums to the blood as amount of such antibodies is declined with time and animal develops its own antibodies in response to gain immunity against infection. On the other hand, ASA in heifers exhibiting 1 AI at pre conception may be only due to cross reactivity and those exhibiting 2 - 3 AIs may be also due to ASA developed against spermatozoa.

Cows

Number of IgG / IgA latex particles attached to the head, tail or both of spermatozoa in the presence of serum of cows was > 5 (Fig 1c). A significant ($P < 0.05$) decline of 17.7 %, 24.46 %, 2.47 % and 22.64 %, 22.28 % , 11.54 % in spermatozoa bound to IgG and IgA latex particles was noticed from 1st post-partum to pre-conception in the presence of blood serum of cows receiving 1 - 3, 4 - 6 and > 6 AIs (Table 1). Milovanovic *et al.* (2005) also observed a high level of ASA in the serum and cervical mucus of cows with longer open day period. A significant ($P < 0.05$) difference was observed in the percentage of spermatozoa bound to IgG and IgA latex particles in the presence of serum of cows exhibiting 4-6 AIs and > 6 AIs at the time of pre conception (Table 1). A significant ($P < 0.05$) continuous decline was recorded in percent positivity from 1st post-partum estrous to pre-conception, which was 21.07%, 28.40% and 39% in cows receiving 1-3, 4-6 and > 6 AIs, respectively (Table 2). Percent positivity for ASA in serum of cows was higher in cows

repeating > 6 times than those repeating 1 - 3 and 4 - 6 times. Fayemi *et al.* (2005) were of the opinion that mean age at 1st calving and mean inter-calving interval were significantly higher ($P < .001$) in the cows positive for ASA compared to negative animals. Sarma *et al.* (2009) observed a titre of 1: 3120 and 1: 1280 in blood and cervical mucus of cows repeating 3-5 times which was higher than normal breeding cows. Recently, Jarora *et al.* (2016) did a comparison in mean values of different immune parameters between total cows (TC) and non-pregnant cows (NPC) which revealed significant rise in reactive sperm percent in serum by NPC than in TC. It can be predicted that the presence of ASA in cows may be due to cross reactivity of different antigens with the same antibodies or specific antibodies generated against the spermatozoa due to repeated inseminations.

Calves, Heifers and cows with ASA of significance

In the indirect SpermMar Test, the occurrence of 40 % or more reaction between the coated latex particles and motile spermatozoa is the lower limit of significant activity. Therefore, percentage of calves (0 days, 365 days), heifers (1st-, pre-conception- estrous) and cows (1st post-partum-, pre-conception estrous) with > 40 % or more reaction between the coated latex particles and motile spermatozoa was calculated. Percentage of calves with > 40 % or more reaction between the coated latex particles and motile spermatozoa was 80 and 70 at the age of one month which was further increased to 100 and 90 at the age of 365

days (Fig 2). Lazarevic *et al.* (2002) also reported an increase in number of calves positive for ASA with advancing age. The increase in percentage of calves with > 40 % IgG- and IgA-ASA may be due to developing defense mechanism against general infections.

Percentage of heifers with occurrence of 40 % or more reaction between the IgG / IgA coated latex particles and motile spermatozoa was 66.7 / 55.5 and 77.8 / 77.8 at the time of 1st estrous in the animals exhibiting 1 AI and 2 - 3 AIs respectively. Percentage of animals exhibiting one and 2 - 3 AIs was reduced to 22.2 / 22.2 and 11.1 / 33.3 for IgG / IgA from 1st estrous to pre-conception. This indicates that percentage of heifers exhibiting 2 - 3 AIs with ASA of significance was higher than those receiving 1 AI. In spite of presence of significant level of ASA in serum of one set of animals (1 AI), fertility was not delayed. But significant level of ASA in serum of another set (2 - 3 AI) of animals delayed the fertility for 2 - 3 cycles which may be due to individual variation (Fig 2).

Percentage of cows exhibiting 1 - 3, 4 - 6, > 6 AIs with > 40 % or more reaction between the IgG and IgA coated latex particles and motile spermatozoa was 80, 90.9, 100 % and 100, 81.81 and 75 % at the time of 1st post-partum estrous respectively. Percentage of animals exhibiting 1 - 3, 4 - 6, > 6 with > 40 % or more reaction between the IgG and IgA coated latex particles and motile spermatozoa was reduced to 40, 9.09, 75 and 20, 9.09 and 50 % from 1st post-partum estrous to pre-conception, respectively (Fig 2).

Percentage of animals with significant level of ASA was high (75 / 50 %) even after repeating >6 times. But all these cows became pregnant at the time of compilation of data. Therefore conditions like cystic ovaries, infectious diseases and other factors may act synergistically with ASA and delayed the fertility. It was observed that when ASA level goes close to or below the level of significance (≤ 40 %) in serum of animals, they conceived. Therefore this study reveals that high level of ASA in serum of heifers and cows delays the fertility for some time.

Effect of ASA on *In vitro* Capacitation and Acrosome Reaction of Cattle bull Spermatozoa

Various stages of acrosome reaction observed during the incubation of cattle bull spermatozoa in energy Tyrode albumin lactate pyruvate medium or TALP supplemented with serum of calves, heifers and repeat breeder cows are shown in (Fig 3). Supplementation of 1 : 50 diluted blood serum of calves, heifers and repeat breeder cows to TALP significantly ($P < 0.05$) declined the percentage of capacitated and viable spermatozoa to 12.01 ± 2.16 %, 14.35 ± 1.84 %, 14.32 ± 2.0 % and 68.60 ± 1.88 %, 57.46 ± 1.62 %, 55.73 ± 1.04 %, respectively (Table 3). On the other hand, an increase in acrosome reacted spermatozoa was observed in the presence of serum of calves (40.1 ± 6.1 %), heifers (44.7 ± 3.7 %) and repeat breeder cows (37.5 ± 1.1 %) as compared to control (33.2 ± 8.0 %). This increase was significant ($P < 0.05$) only in the presence of serum of heifers. Thus ASA in serum enhanced the rate of *in vitro* acrosome reaction of cattle

bull spermatozoa. But rate of overall capacitation/ acrosome reaction was higher in the presence of serum in comparison to control. It may be due to increase in membrane fluidity, because capacitation is associated to increased membrane fluidity caused by the removal of cholesterol from sperm plasma membrane via sterol acceptors present in the female tract secretions (de Lamirande *et al.*, 1997). Since locally produced secretory immunoglobulins occur in the genital tract in addition to serum-derived Ig (Rumke, 1974), therefore, premature acrosome reaction can be expected *in vivo*. It is established that spermatozoon binds to the zona pellucida (ZP) with its intact plasma membrane after penetrating into the cumulus oophorous. Sperm binding occurs via specific receptors to ZP glycoproteins located over the anterior sperm head (Breitbart *et al.* 1997). The acrosome reaction is a stimulus-secretion coupled exocytotic event in which the outer acrosome membrane fuses with the overlying plasma membrane (Yanagimachi *et al.*, 1994). The acrosome reaction (AR) seems to be physiologically induced by natural stimulants such as the follicular fluid (FF), progestin, progesterone, and hydroxy progesterone (Mansour *et al.*, 2008). Follicular fluid and cumulus cells have protein-bound progesterone that has been identified as one of the most important acrosome reaction-inducing agents (Brucker *et al.*, 1995). Therefore, premature acrosome reaction in the presence of ASA/cross-reacting antibodies in uterine fluid before reaching the oocyte may impede fertilization. A variation in percentage of capacitated, acrosome

reacted and viable spermatozoa were also observed in the presence of serum of calves, heifers and repeat breeder cows. It may be due to difference in level of ASA among the animals as observed by ELISA and Sperm Mar test.

MDA production/10⁹ spermatozoa in capacitated/acrosome reacted spermatozoa was 14.87 ± 0.66 in control. It was enhanced to 37.27 ± 6.54, 17.25 ± 0.98 and 25.23 ± 5.08 in the presence of serum of calves, heifers and repeat breeder cows (Table 3). Increase in MDA production/10⁹ spermatozoa was significant (P < 0.05) only in the presence of serum of calves and cows. It revealed that due to increase in rate of acrosome reaction, generation of reactive oxygen species (ROS) was also increased. Spermatozoa itself produce small amounts of ROS that are essential to many of physiological process i.e. capacitation, hyperactivation and sperm oocyte fusion (Aitken *et al.* 2003). Low level of ROS has also been shown to be essential for fertilization, acrosome reaction and motility. It is possible that binding of ASA/ cross-reacting antibodies to sperm membrane during incubation in TALP supplemented with serum enhanced acrosome reaction leading to generation of more reactive oxygen species.

A lot of work has been done in human to find out the effect of experimentally generated or naturally produced ASA on the process of fertilization, but reports are contradictory. Shibahara *et al.* (1996) investigated the blocking effects of complement-dependent sperm immobilizing antibodies in the sera of infertile women and

monoclonal antisperm antibodies against humans and mice on fertilization. They were of the opinion that some of them may inhibit sperm capacitation and thus prevent all processes of fertilization that follow. Some other antibodies may not affect capacitation and sperm binding to zona pellucida but inhibit the acrosome reaction, followed by the blocking of sperm penetration through zona pellucida and ooplasm. Francavilla *et al.* (1997) were of the opinion that IgG-ASA transuded

from the blood into the genital tract can exert the inhibitory effect on ZP binding *in vivo*. During the present study ASA/cross-reacting antibodies in serum of calves, heifers increased the rate of acrosome reaction in comparison to control. Francavilla *et al.* (1999) were of the opinion that sperm-bound antibodies can interfere with sperm functions involved in the fertilization process, mainly in the sperm-zona pellucida interaction.



Table 1: Percentage of IgG and IgA bound spermatozoa in the presence of blood serum of calves, heifers /cows with advancing age and number of inseminations.

Animal	IgG bound spermatozoa		Percentage Increase/ decrease	IgA bound spermatozoa		Percentage Increase/ decrease
	Ist Sample	Last sample		Ist Sample	Last Sample	
Calves (n = 10)	45.10±3.64	47.92±2.53	2.82	44.59±4.22	46.41±1.99	1.82
Heifers						
1 AI (n=18)	49.96±3.46 ^a	37.04±2.02 ^b	12.92	43.87±3.46 ^a	35.90±1.70 ^b	7.97
2-3 AI (n=10)	48.86±3.78 ^a	35.02±1.56 ^b	13.84	51.89±4.93 ^a	36.64±1.70 ^b	15.25
Cows						
1-3AI (n=5)	57.07±3.99 ^a	39.37±4.09 ^b	17.7	58.52±1.38 ^a	35.88±1.00 ^b	22.64
4-6 A.I (n=11)	54.09±3.87 ^a	32.63±2.43 ^{bc}	24.46	53.65±5.87 ^a	31.37±2.67 ^{bc}	22.28
>6 A.I (n=4)	52.18±9.02	49.71±4.03 ^d	2.47	55.31±8.35	43.77±3.49 ^d	11.54

Ist sample: calves (7days old); heifers (Ist estrous) and cows (Ist post-partum estrous) Last Sample: calves (365 days old); heifer, cow (Pre conception)

Different superscripts indicate significant difference at 5 % level with in the columns (a,b) and rows (c,d).

Table 2: Percentage positivity for antisperm antibodies detected by ELISA in the blood serum of Calves, Heifers / Cows with advancing age and number of inseminations.

Animals	Sample no.								Percent decrease
	1	2	3	4	5	6	7	8	
Calves	40.1±5.1 ^{ab}	43.9±4.4 ^{ab}	45.0±5.78 ^a	41.2±4.7 ^{ab}	40.9±4.9 ^{ab}	30.1±5.4 ^{bc}	22.3±3.6 ^{cd}	16.4±1.5 ^d	23.7
Heifers									
One A.I (n=8)	45.9±6.6	40.7±4.9							5.2
2-3 A.I(n=10)	36.0±5.8	32.2±4.9							3.8
Cows									
1-3 A.I (n=7)	48.9±5.6 ^a	37.7±4.4	27.9±3.3 ^b						21.1
4-6 A.I (n=10)	55.8±3.4 ^a	43.5±4.5 ^b	33.7±2.4 ^b	27.4±2.1 ^b					28.4
>6A.I (n=3)	60.3±12.7	55.0±11.8	62.7±15.4	29.7±5.4	27.3±4.7	21.3±9.4			39

Calves: Sample 1-8 (7 days to 365 days of age at 45 days interval)

Heifers: Sample1 (Ist estrous), Sample 2 (Pre conception)

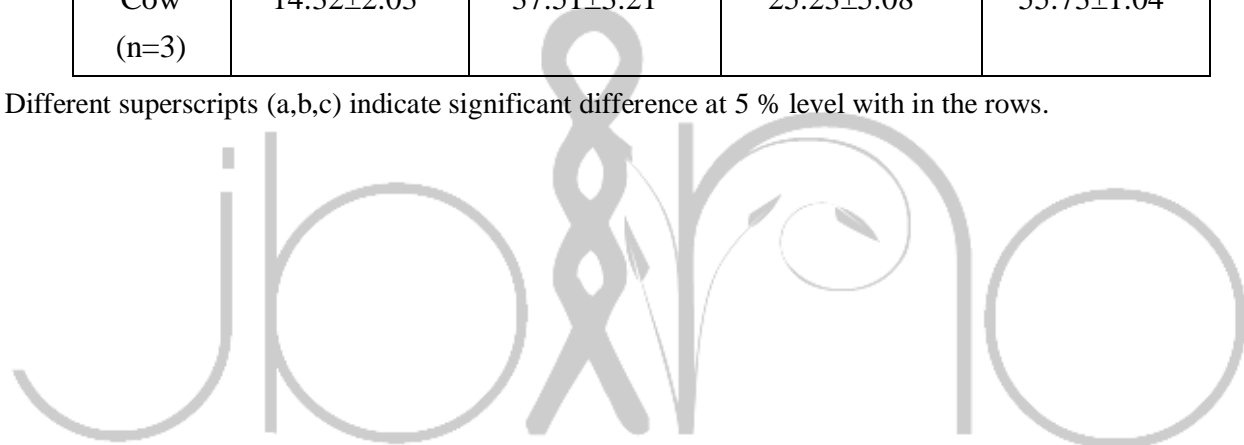
Cows: 1-3AIs:-Sample 1 (Ist post-partum estrous), Sample 2 (Pre conception), 4-6 AI: Sample 1-4 (Ist post-partum till Pre conception) and >6 A.I: Sample 1-6 (Ist post-partum estrus till Pre conception)

Different superscripts (a,b) indicate significant difference at 5 % level with in the columns

Table 3: Effect of blood serum of calves, heifers and cows on *in vitro* capacitation and acrosome reaction of cattle bull spermatozoa.

Group	Capacitated spermatozoa	Acrosome reacted spermatozoa	MDA production/10 ⁹ spermatozoa	Viable spermatozoa
Control (n=3)	28.08±0.84 ^a	33.56±2.94 ^a	14.87±0.66 ^{ab}	85.78±1.01 ^a
Calf (n=3)	12.01±2.16 ^b	40.58±4.17 ^{ab}	37.27±6.54 ^c	68.60±1.88 ^b
Heifer (n=3)	14.35±1.84 ^b	44.7±3.11 ^b	17.25±0.98 ^a	57.46±1.62 ^c
Cow (n=3)	14.32±2.03 ^b	37.51±3.21 ^{ab}	25.23±5.08 ^{bc}	55.73±1.04 ^c

Different superscripts (a,b,c) indicate significant difference at 5 % level with in the rows.



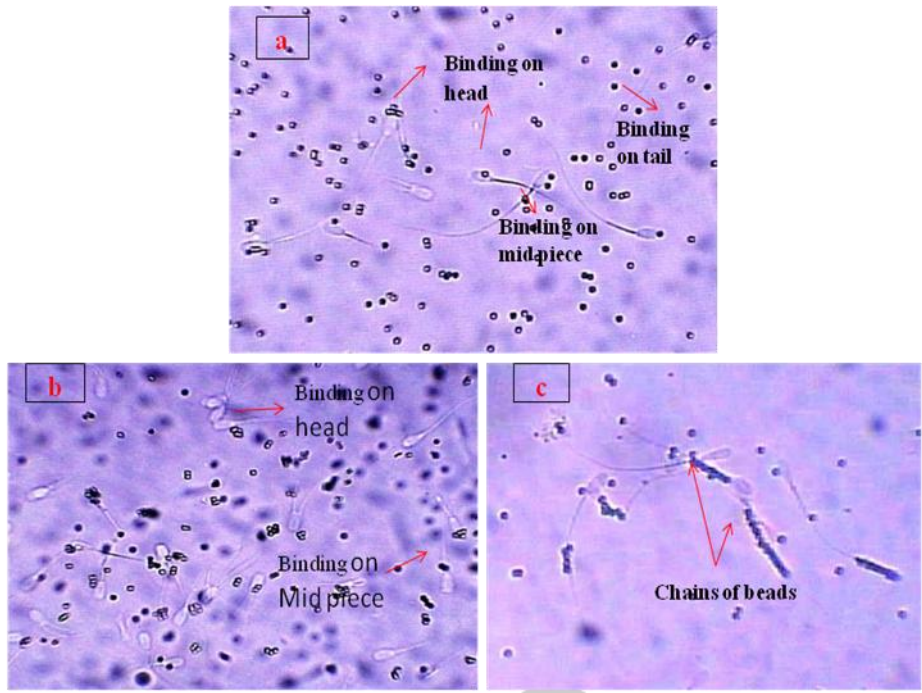


Fig 1: Attachment of latex particles to different parts of spermatozoa in indirect Sperm Mar test in the presence of blood serum of calves (a), heifers (b) and cows (c).

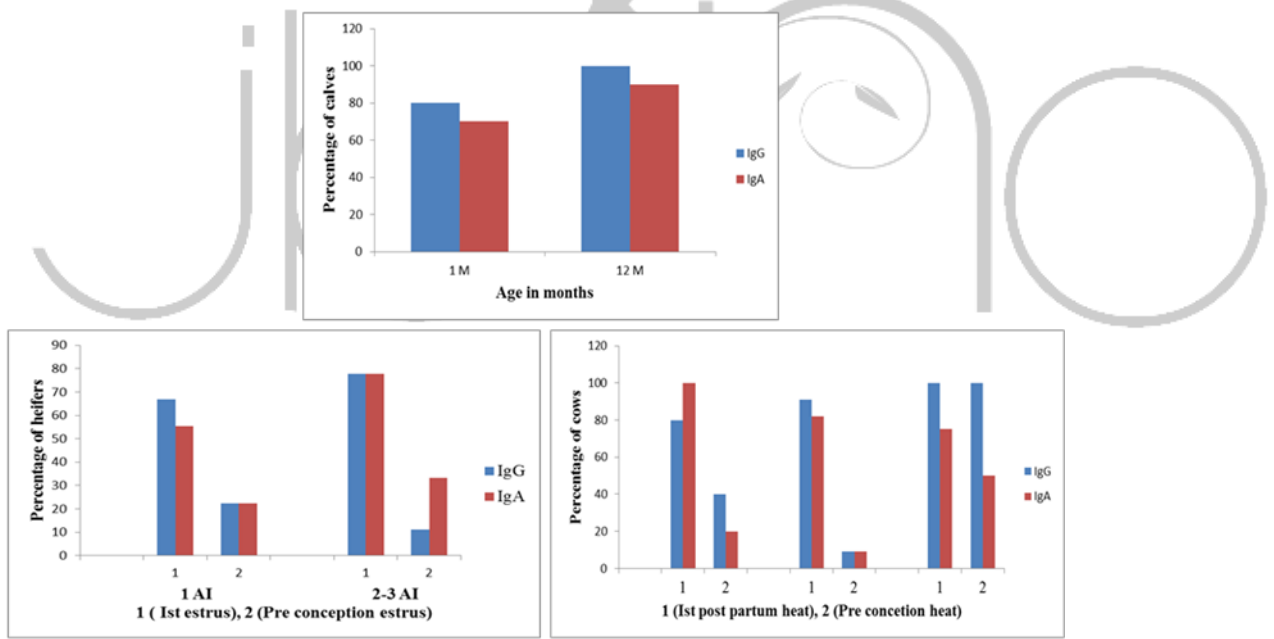


Fig 2: Percentage of calves, heifers and cows with significant level of IgG / IgA-ASA

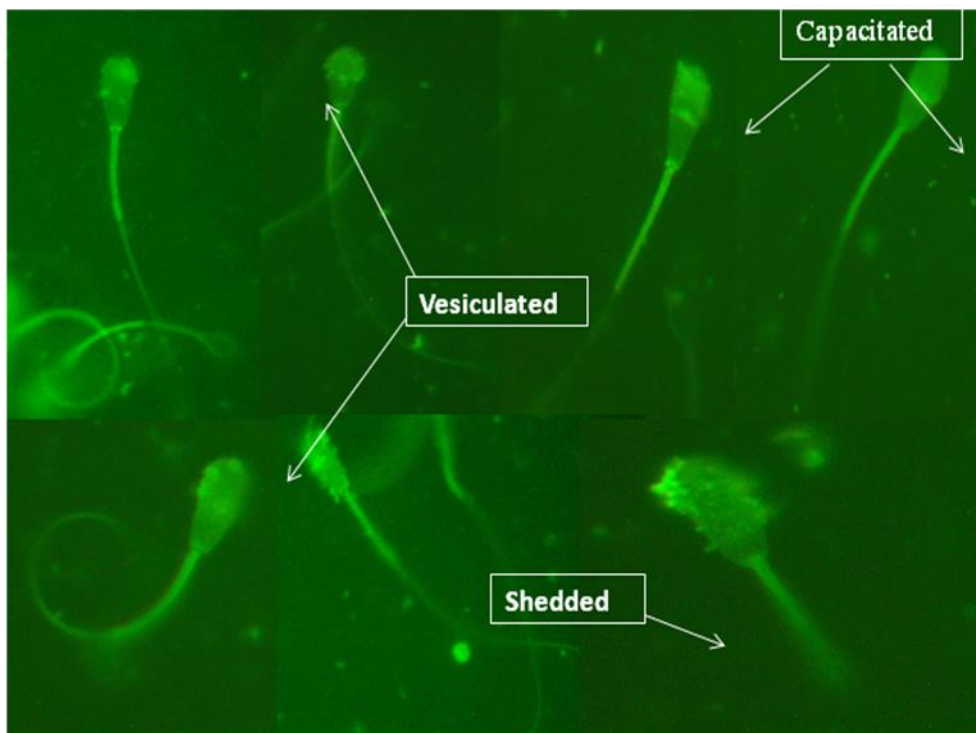


Fig 3: Cattle bull spermatozoa incubated in Energy TALP with or without blood serum of calves, heifers and cows showing capacitated and acrosome reacted spermatozoa (1000X).

Conclusions

The study concluded that naturally occurring developing antibodies in blood serum of calves, heifers and cows are reactive to spermatozoa and delays the fertility in heifers and cows. Higher rate of acrosome reaction observed in the presence of serum may be due to premature acrosome reaction which can affect the fertilizing ability of sperm.

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