Detection of Antisperm Antibodies in Serum of Repeat Breeding Cows

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Introduction

- Repeat Breeding, an important cause of low reproductive efficiency where animals fail to conceive after 3/more A.I in absence of detectable reproductive abnormalities.

Theoretically-

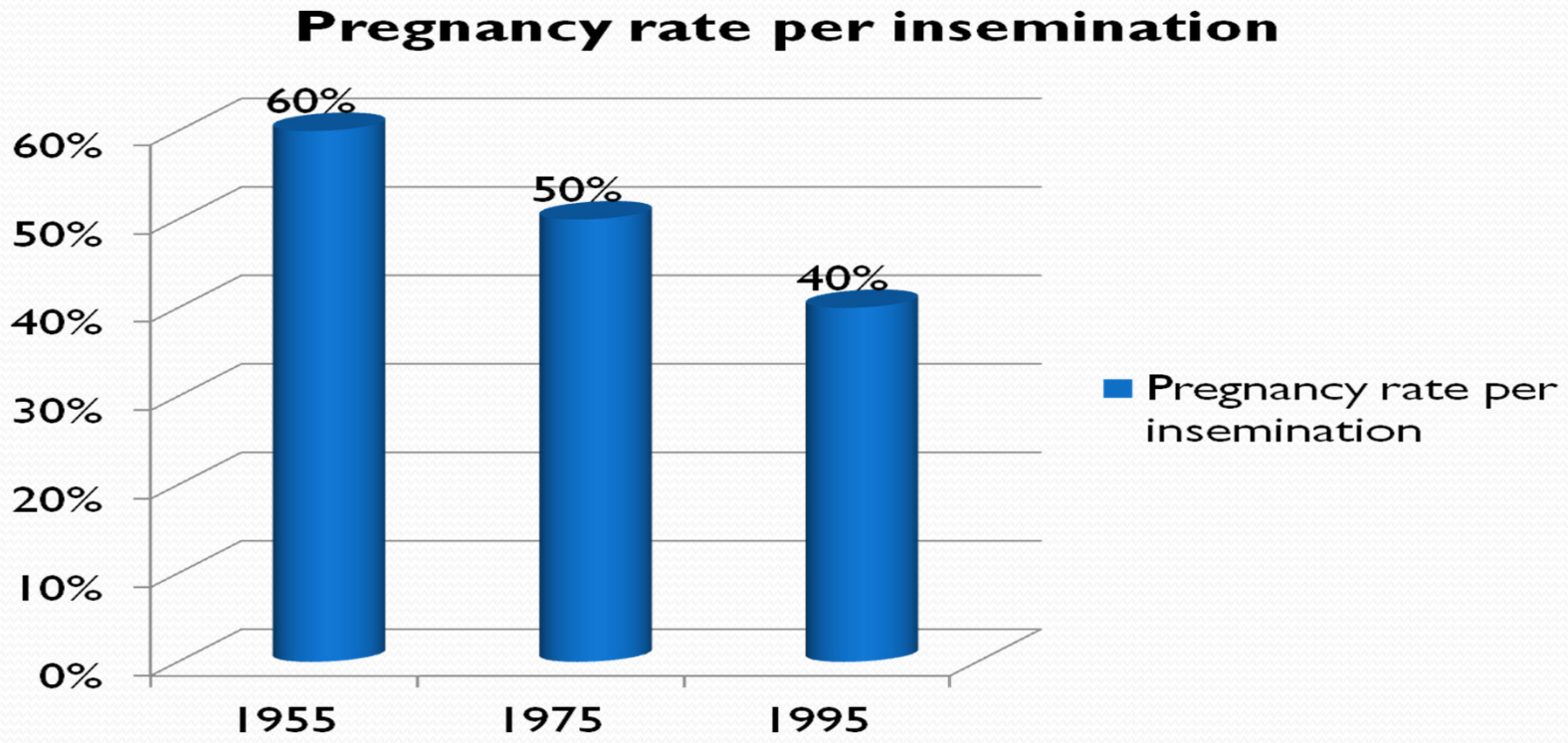
Ideally, if conception rate is 60%, R.B should not exceed 5%

Presently, the incidence is higher (25-30%)

Warrants investigation..

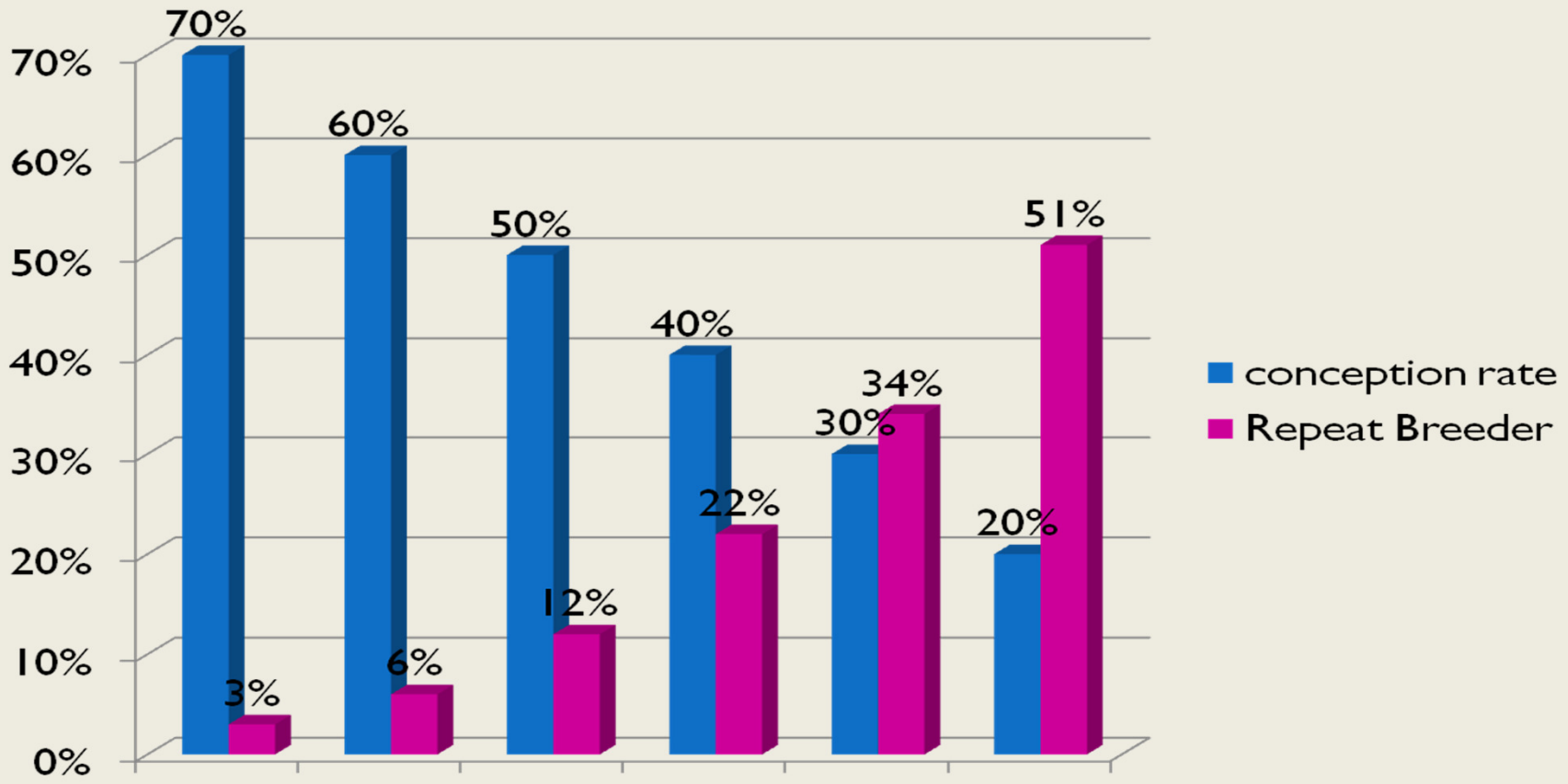
Scope for the research in R.B

1. Declining trend of pregnancy/conception rate



(Wiltbank, 1998)

2. Relationship b/w conception rate and R.B

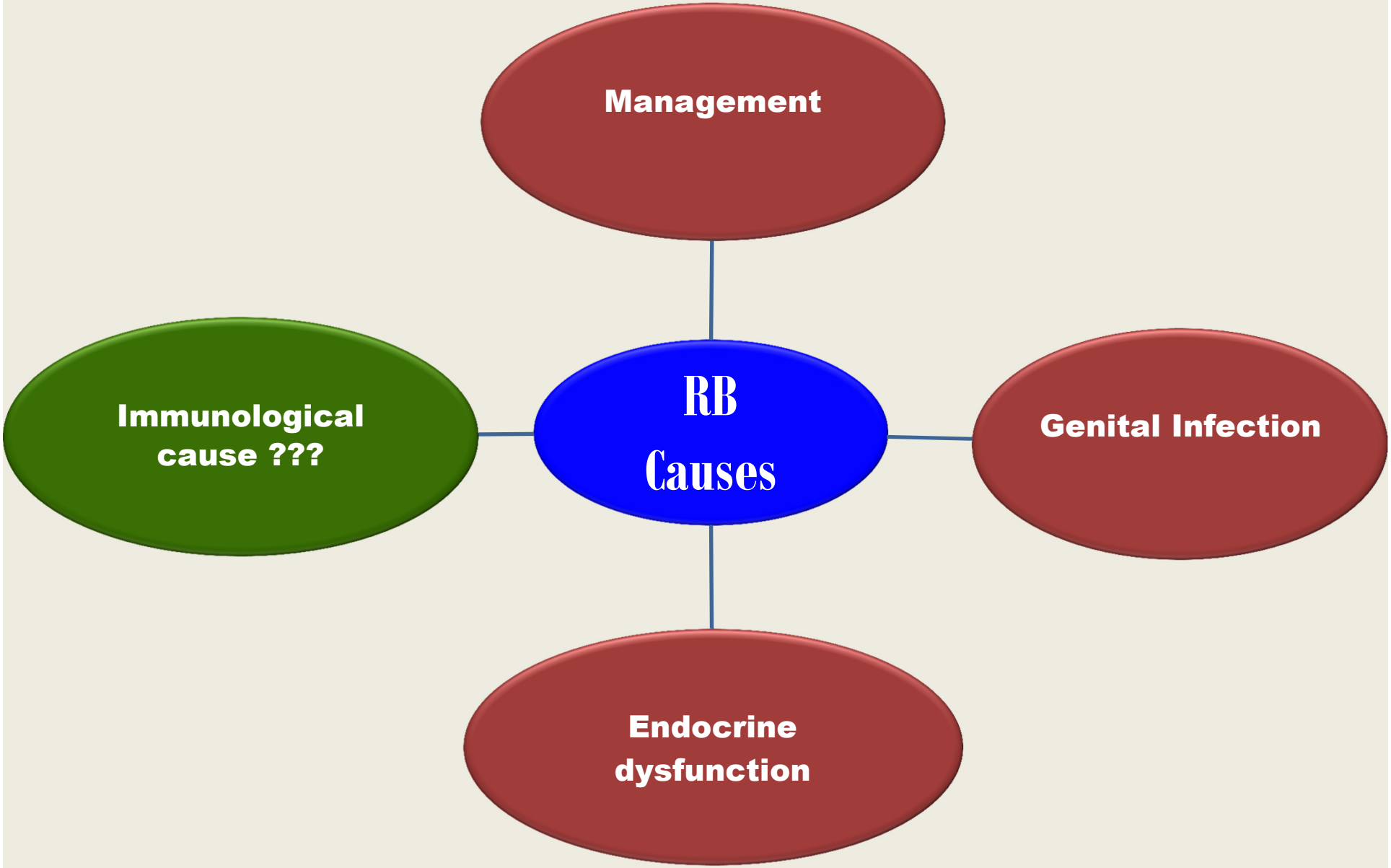


(Reneau & Conlin, 1984)

Incidence of Repeat Breeding in India

Reference	Region	Incidence %
Kaikini *et al.,* 1983	Maharashtra	21.9 %
Sharma *et al.,* 1991	Chhotanagpur	51.79%
Karwani and Sharma, 2003	Punjab	19.61%
Das *et al.,* 2004	Orissa	38.18
Saxena, 2004	Uttrakhand	33.3%
Butani *et al.,* 2008	Gujarat	45.15%
Bhattacharyya & Buchoo, 2008	Kashmir	27.52%

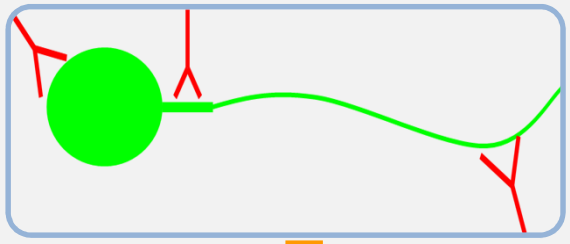
4. Multifaceted causes



Incidence of ASAs

Reference	Sample type	Regular breeder (%)	Repeat breeder (%)
Bhatt *et al.*, 1979	CM	8.3	16.6
	Serum	0	41.5
Cote *et al.*, 1980	Serum	0	64
Seshagiri *et al.*, 1987	CM	---	6.9
	Serum	11.6	35.6
Wang, 1989	CM	0	37.9
	Serum	6.7	34.5
Wang & Xie, 1990		4.9	39.5
Risvanli *et al.*, 2003	Serum	7.5	17.6
Sarma *et al.*, 2009	CM	-	100 (1:320)
	Serum	-	100 (1:640)

Effect of Antisperm Antibodies (ASA)



Impair metabolic activity of sperm

Progressive sperm motility compromised

Impair capacitation & Acrosome reaction

Decrease ability to penetrate oocyte

Impaired Blastocyst development or implantation

Fertilization failure/ EED

Repeat Breeding or Immuno infertility

(Boring *et al.*, 2001)

Materials and Methods

- Blood was collected from Heifer, Normal Breeder, Repeat Breeder and pregnant cows.
- Serum was separated and tested for specific causes (IBR, Brucellosis) in Ist three groups.
- Cervical Mucus was also collected, tested for nonspecific infection by white side test.
- Animals found **negative** were included in experiment.
- Serum was inactivated at **56^{o}c for 30 min**. to avoid any non-specific binding/inactivate the complement.

Method….

- ASA were detected in serum of all four groups by **Tube Plate Agglutination Test.**
- Serum samples were diluted in PBS at 1:1, 1:5 and 1:10 dilution rate
- Sperm concentration was adjusted as **60 million /ml**.
- **160 µl.** Diluted sera of each group was taken in the wells of micro titer plate
- **40 µl.** Sperm suspension was added to each wells.
- The plate was incubated in water bath at **37^{o} C for 60 min**.

Cont....

- Contents of each well was aspirated and mounted on glass slides

- Slides were examined by phase contrast microscopy under **40X** objective.

- No. of **clumped** and **un-clumped** spermatozoa were counted in five high power field.

- Percent agglutinated spermatozoa were calculated.

Results

Table 1. Sperm agglutination in serum (1:1 dilution)

Group	N	Agglutinated sperm Mean±SE	Range	% Agglutination	Range
Heifer	14	8.71 ± 1.42[a]	4-18	10.74[a]	4.76-13.33
Normal breeder cows	16	18.62 ± 1.33[b]	10 -35	21.28[ab]	14.85-31.86
Repeat breeder cows	9	**26.33 ± 1.77[c]**	19-33	**31.46[b]**	27.58-38.82
Pregnant cows	16	14.06 ± 1.33[b]	4-25	15.20[ad]	8.33-19.84

Mean with different superscript differ significantly **(p<0.01)**

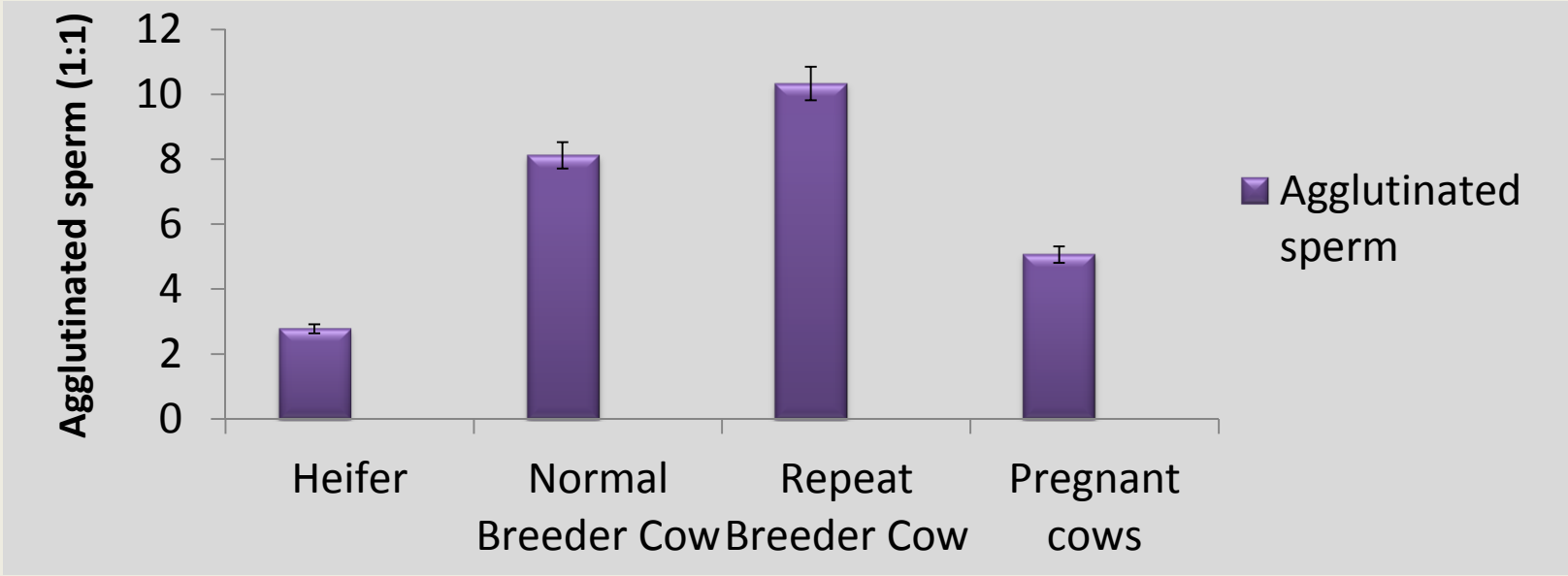


Fig-1a: Agglutinated sperm in serum (1:1 dilution)

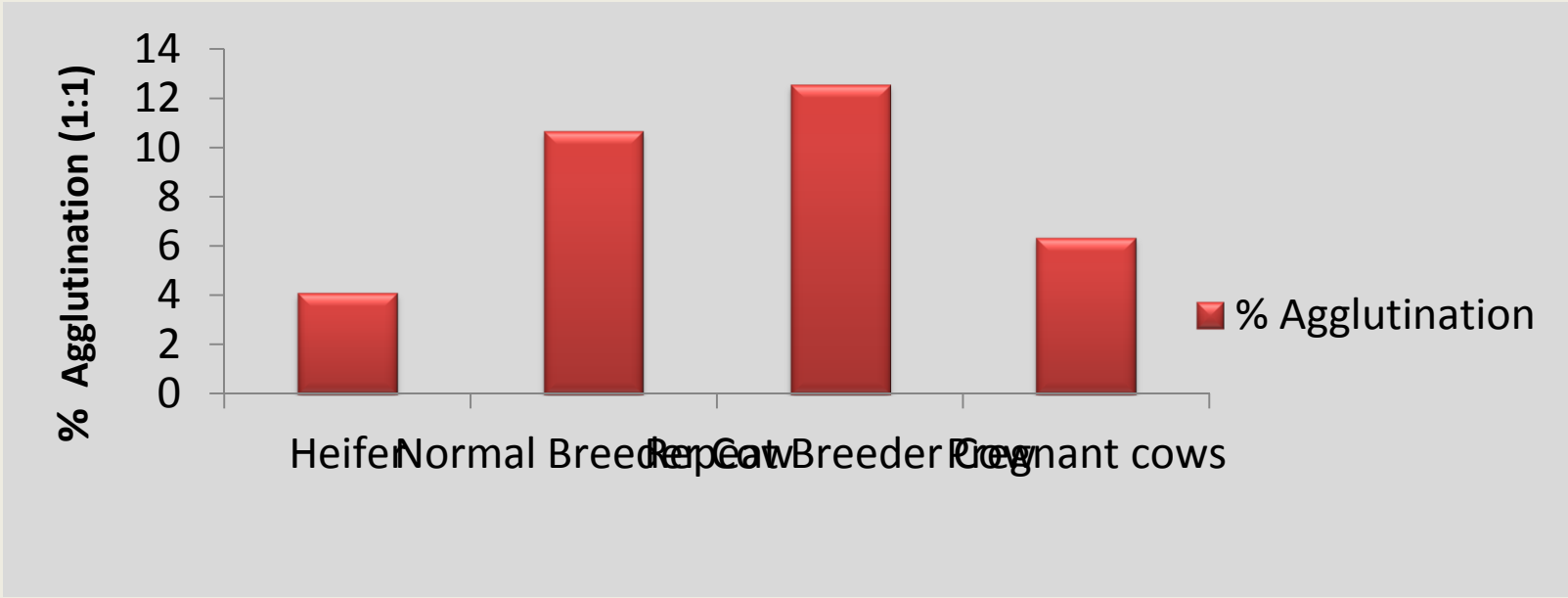


Fig-1b: Percent agglutination in serum (1:1 dilution)

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Table 2. Sperm agglutination in serum (1:5 dilution)

Group	N	Agglutinated sperm Mean±SE	Range	% Agglutination	Range
Heifer	14	4.36 ± 0.82^{a}	2- 8	6.50^{a}	4.08-8.57
Normal breeder cows	16	10.37 ± 0.77^{b}	6 -16	14.70^{bc}	10.41-21.53
Repeat breeder cows	9	**16.44 ± 1.03^{c}**	8- 24	**19.31^{b}**	15.68-24.48
Pregnant cows	16	8.06 ± 0.77^{b}	5-15	9.38^{adc}	6.12-13.27

Mean with different superscript differ significantly **($p<0.01$)**

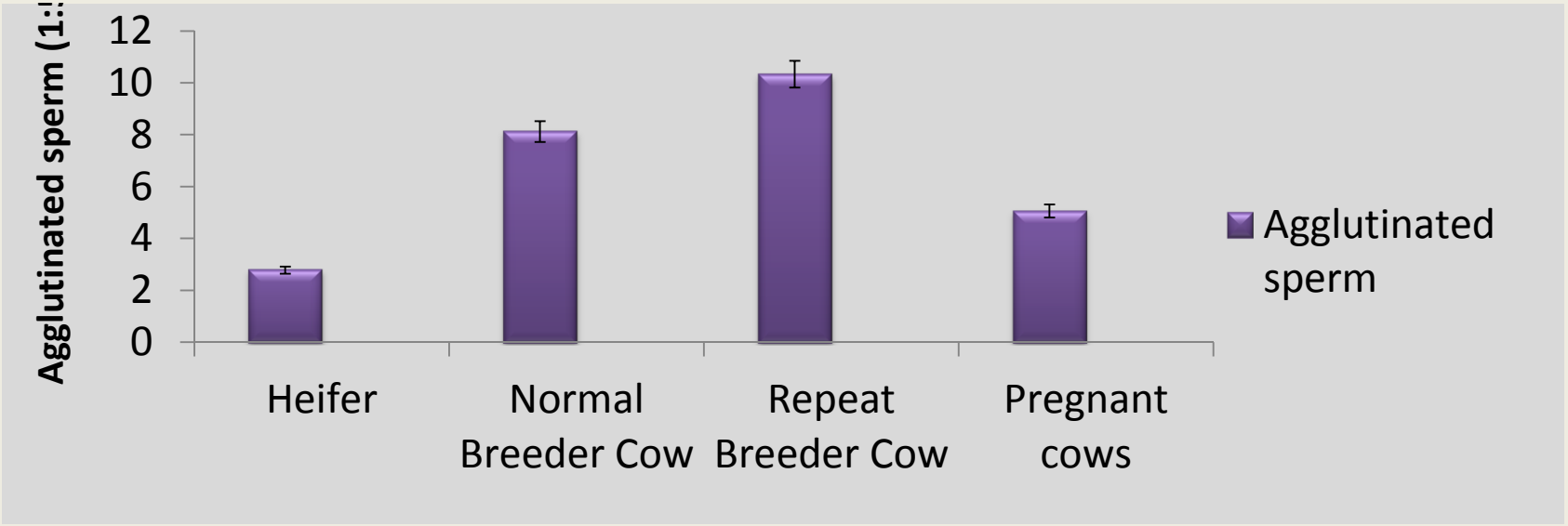

Fig-2a: Agglutinated sperm in serum (1:5 dilution)

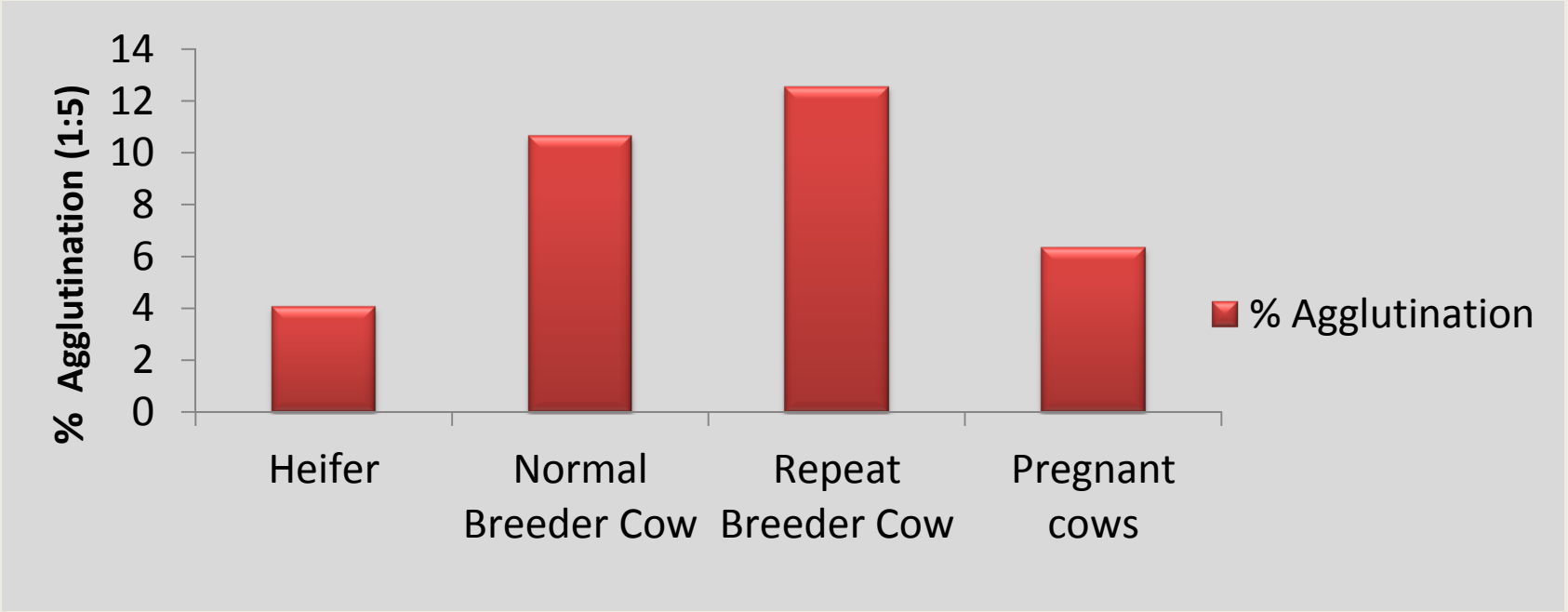

Fig-2b: % Agglutination in serum (1:5 dilution)

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Table 3. Sperm agglutination in serum (1: 10 dilution)

Group	N	Agglutinated sperm Mean±SE	Range	% Agglutination	Range
Heifer	14	2.78 ± 0.50[a]	2- 4	4.06[a]	2.50 - 5.55
Normal breeder cows	16	8.12 ± 0.47[b]	5 -11	10.66[b]	7.36 - 11.95
Repeat breeder cows	9	**10.33 ± 0.63[c]**	7- 18	**12.56[b]**	9.63 - 17.14
Pregnant cows	16	5.06 ± 0.47[d]	3- 8	6.33[ad]	4.12 - 8.62

Mean with different superscript differ significantly **(p<0.01)**

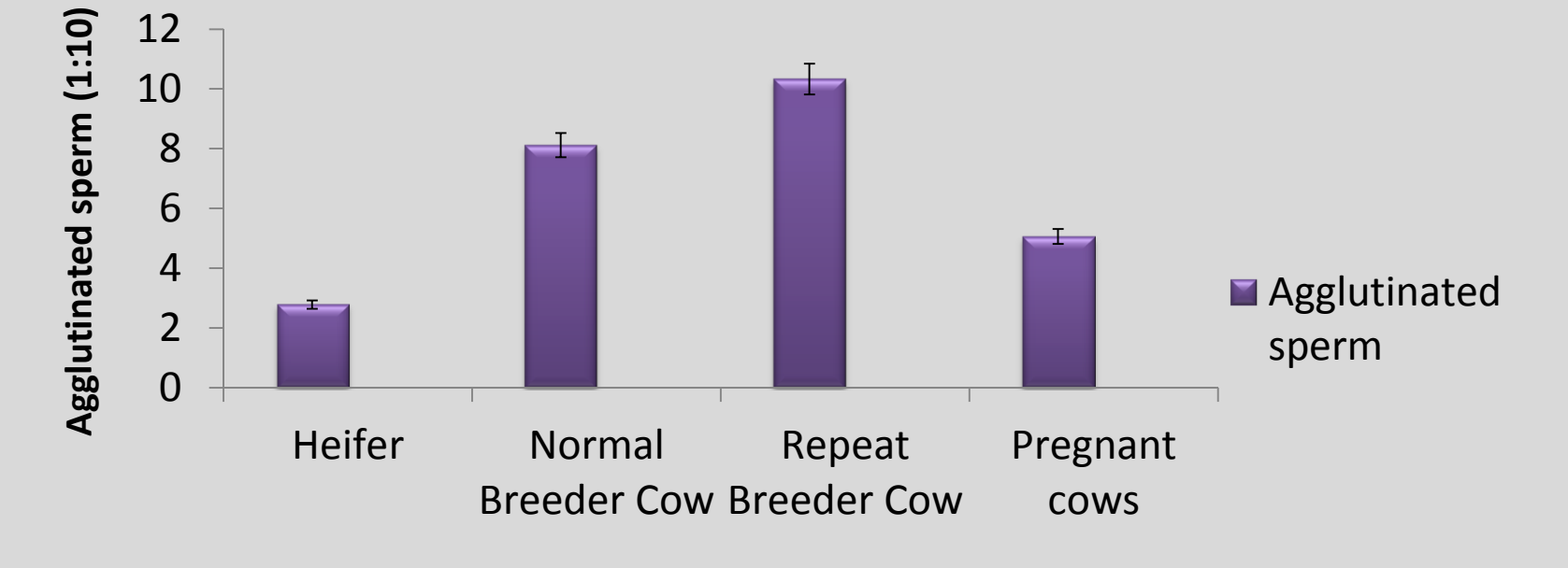


Fig-3a: Agglutinated sperm in serum (1:10 dilution)

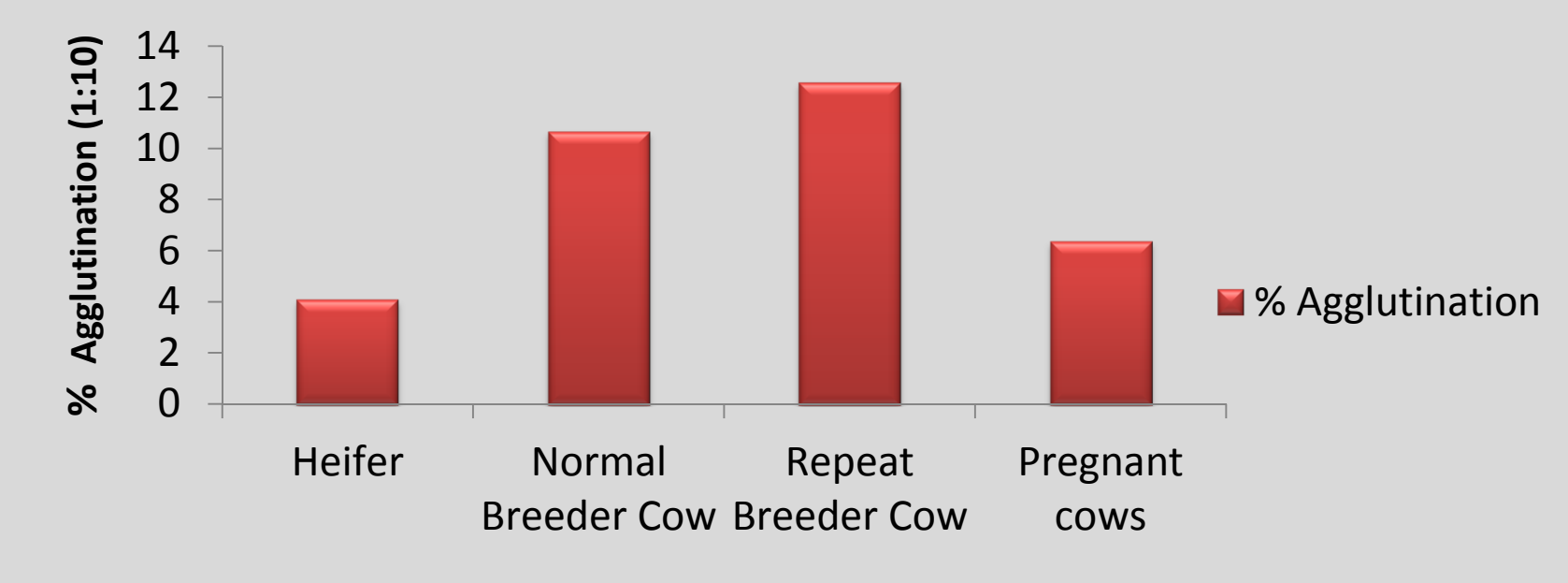
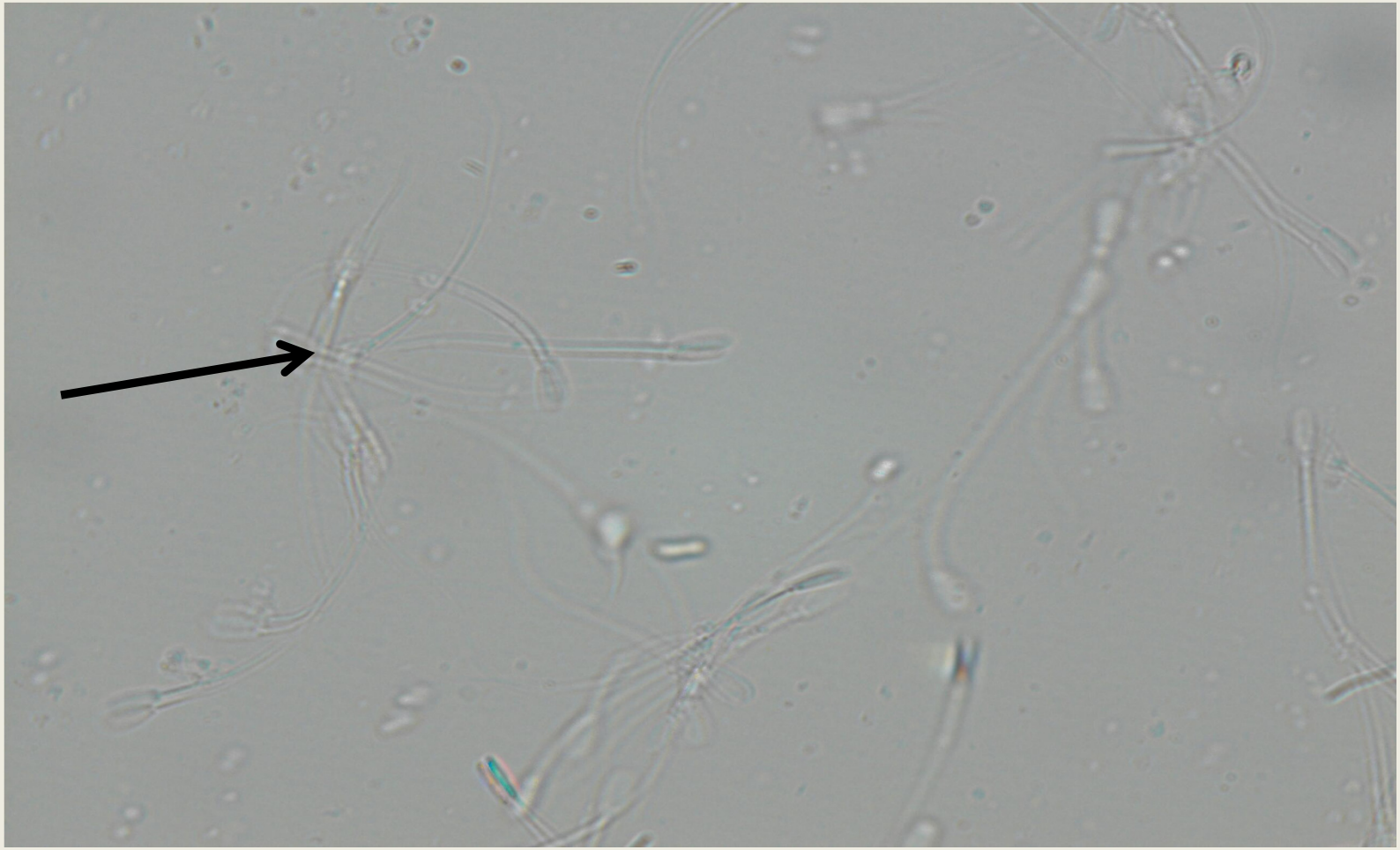
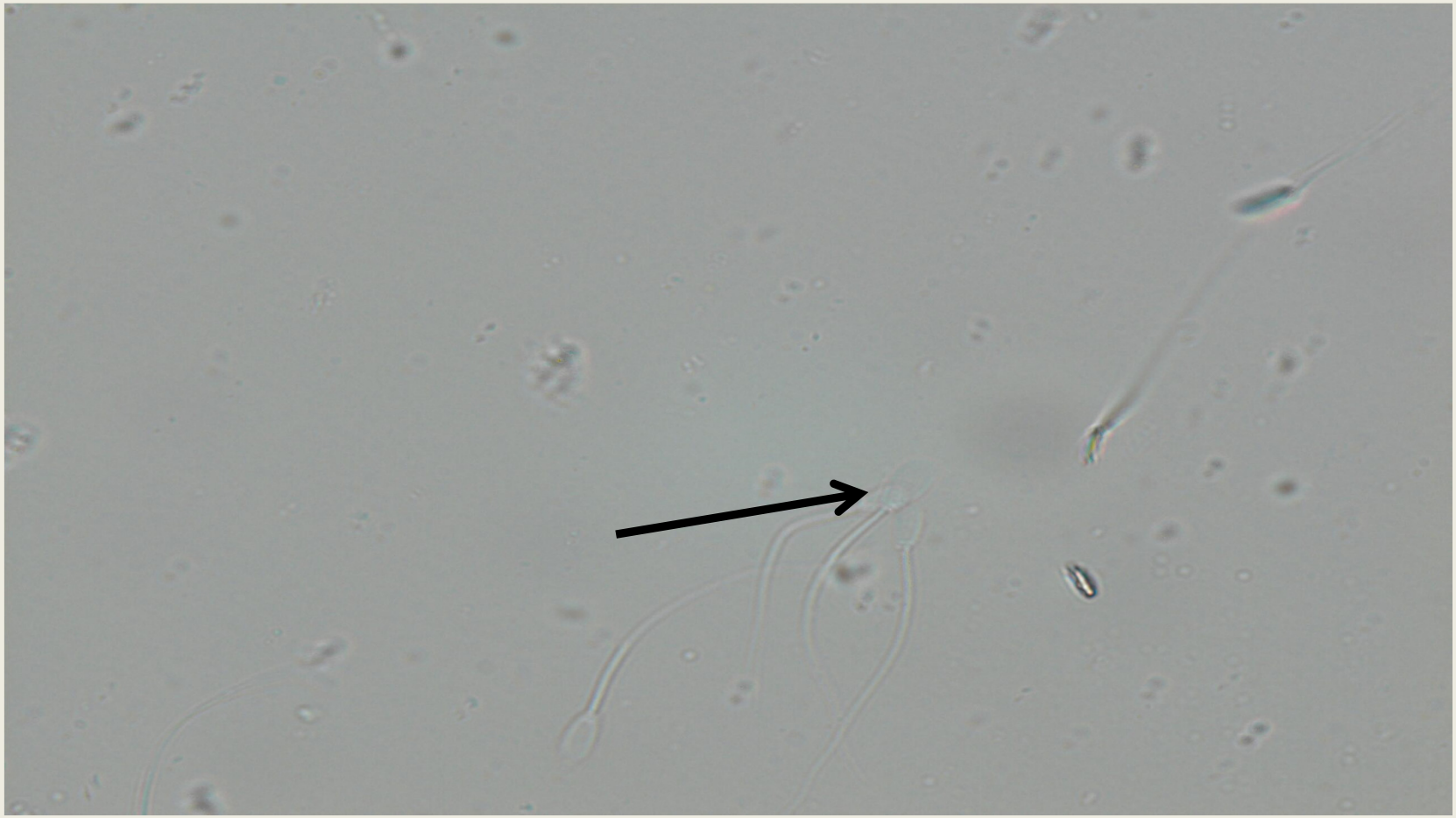


Fig-3b: % Agglutination in serum (1:10 dilution)



→ **Head-Head clumping of spermatozoa**



→ **Head-Head clumping of spermatozoa**

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Conclusion:

- Presence of 31.46; 19.31and 12.56% sperm agglutination in 1:1; 1:5 and 1:10 serum dilution respectively indicates that antisperm antibodies may be a cause repeat breeding in cattle

Thank You