Viability Spermatozoa Epididymis of Buffalo (*Bubalus Bubalis*) In Fertilized Media to Additional Serum at Temperature 5°C

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Abstract. This study purposed to determine the quality of Buffalo’s epididymis Spermatozoa on the TALP (Tyrode Albumin Sodium Lactate Sodium Pyruvate) fertilization at the 5°C temperature. The Randomized Block Design used on this research with 3 treatments and 5 blocks as replication. The treatments were additional serum: P1 (0%), P2 (10%) and P3 (20%). The results showed that the highest motility was on the treatment P3 (20%), about 66.00±9.45 was significantly effect (P<0.05) than P1 and P2. The highest percentage of live on P3 (20%) was 75.20±2.41 and significantly effect (P<0.05) than P1 and P2. The best percentage of abnormality was also on the P3 (20%) i.e.14.20±1.30 significantly effect (P<0.05) compare P1 and P2. It could be concluded that the addition of 20% serum in TALP media as a fertilization medium can improve the buffalo’s spermatozoa quality of caudal epididymis.

1. Introduction
Swamp Buffalo (*Bubalus Bubalis*) is the cattle that grown extensively in west Sumatera for their meat production. The increasing rate of Swamp Buffalo slaughtering will become threat of extinction in the future. Every pair of testis and epididymis of three years old Swamp Buffalo is a source of genetic material that can be used to preserve buffalo trough reproduction technology. Swamp Buffalo Bull Epididymis is a source of material genetic that hat can be stored for long period [1]. One of the part Epididymis, especially caudal is a sources of spermatozoa that has capability to fertilize as same as ejaculated spermatozoa [2].

In some cattle, the effort to safe genetic material from epididymis through spermatozoa processing (Liquid and Frozen) has been done with good result, African Buffalo [3]; [4] and Strip Buffalo [5]; [6] are one of them. According to [7], the processing of spermatozoa from epididymis becomes an alternative method that can be applied to cattle that has superior genetic quality. The damaged that spermatozoa gets when preservation at low temperature is a major problem in maintaining the quality of the cement. By using TALP medium (Tyrode Albumin Sodium Lactate Sodium Pyruvate) and adding other material such as serum will overcome those problem. Since the beginning the use of TALP medium is still limited for fertilization in vitro.

TALP medium is very beneficial because it has a lot of nutrient liked NaCl, CaCl, MgCl, NaH2Po4, NaHCO3, Heps, Piruvat Acyd, Gentamicin and Caffeine. Meanwhile serum can improve the quality of sperm as stated by [8] blood serum contain glucose, fat, Non protein substance, Nitrogen, Enzyme, Hormone, Vitamin and Pigment. Plasma protein consist of 90% water and 10% solid. These
Solid contains 7% protein such as antibody, phospholida cholesterol, and glucose, enzyme meanwhile inorganic non protein comprises P, Na, Ca, K, Mg, Fe and HCO3. Furthermore serum also contains hormone such as estradiol and estrogen that can produce nutrition for spermatozoa [9]. And then by adding serum into TALP medium can increase percentages viability of spermatozoa 84% and spermatozoa motility 72% and decrease abnormal spermatozoa 9,66% [10].

By adding serum in TALP medium that collected from caudal epididymis of Swamp Buffalo at temperature 5°C. It is expected that with the addition serum inti TALP medium will minimalize the damaged to spermatozoa during preservation at 5oC, so by that the spermatozoa can be kept longer. Based on that. This research was aimed to study the effect of TALP medium with addition serum treatment on the quality of spermatozoa from caudal epididymis of Swamp Buffalo bull after preservation at 5°C temperature.

2. Material and Methods
Caudal epididymis of buffalo will be collected in slaughterhouse in Padang city when slaughter time. Caudal epididymis was washed and stored in physiological solution (NaCl 0. 9%) as transportation media. Then sperm was collected from caudal epididymis trough slicing technique and emphasis each tissue of caudal [11] with TALP medium as diluent. Fresh spermatozoa (before diluted) from caudal epididymis will be evaluated likes spermatozoa concentration, motility, viability and abnormal spermatozoa.

Haemositometer and number chamber can be used to calculate spermatozoa concentration. Spermatozoa of undiluted collection are placed on top of the object glass then sucked with erythrocyte pipette up to 0.5 and adding NaCl 3% until reach 101 and then homogenization. The concentration will be calculated from five chamber of neubauer [12]. The percentage of progressive motility of spermatozoa was counted objectively on 8 different field of view with a 400 X magnification microscope [13]. The number will be ranged between 0% until 100% with 5% scale. Viability of spermatozoa was determined by using eosin B (Merck,Cat. No 5009 K50003834, Germany) [12]. The Characteristic of living spermatozoa is having transparent head, while the dead spermatozoa has red head. At least 200 sperm that have been evaluated using 400X microscope. Percentages of abnormal spermatozoa that does not have head or tail, having big head or small, having to head or two tail [12].

The collected spermatozoa are then centrifuged at 25000 rpm for 5 minutes at room temperature. The forming supernatant is discarded and the spermatozoa containing sediment is rediluted with a diluent material adjusted to the treatment. Spermatozoa from cauda epididymis is diluted by TALP medium. P1 (control) use TALP medium without addition of serum diluted with the ratio of 1:4, at P2 TALP medium with addition of 10% serum and at P3 TALP media with addition of 20% serum treatment. Observation will be done after 4 hours preservation in liquid form at temperature 5°C. The evaluated variable in this study is the quantity and quality of fresh spermatozoa after collecting concentration, motility, viability and abnormality. The collected data will be analyzed with multiform method (ANOVA) in form of randomized block design with three treatments of serum addition in TALP medium and 5 time repetition. The difference of each treatment were tested using the smallest significant difference test technique [14].

3. Result and Discussion

3.1. Quality of fresh epididymis spermatozoa
The concentration of spermatozoa that obtained was 123,2 x 107 spermatozoa/ml. This result was still below the standard range mentioned by [5] that the concentration of spermatozoa in cauda epididymis of mammals is 10.000-100.000 million cell/ml. According to [15] cauda epididymis of Garut sheep had a concentration of spermatozoa above 13.993,33 million cell/ml (range between 13.530 until 14.520 million cell/ml). The different of the result caused by the different type of cattle and type of collected method. The percentage mortality of spermatozoa from fresh epididymis of swamp buffalo result from this research (80±5.22%) was more higher than the research from [16], [4] about spermatozoa from cauda epididymis of African Buffalo (Syncerus caffer) that is 60,0±3,82%, 58±17% and 53,0±12,51%.
The different of the result also caused by the difference of buffalo species and the condition of each individual of buffalo bull that used in the research. The viability of fresh spermatozoa epididymis in this research was 85.5±2.12% more lower than stated by [16] to spermatozoa epididymis from African buffalo that is 92.75±2.25%. Differences in the result were due to different type of buffalo bull species and the condition of each bull individual that used in the research. The result of abnormal spermatozoa this research got was 21.2±1.64%. Spermatozoa abnormalities were most prevalent in this observation due to the presence of cytoplasmic droplets in the distal portion. However, this result was still in standard range of abnormality spermatozoa that appropriate for fertilization.

3.2. Epididymis spermatozoa quality in TALP media with serum treatment

Table 1. Motility, viability, abnormality epididymis spermatozoa of swamp buffalo with serum treatment after preservation

| Treatment       | Motility    | Viability   | Abnormality |
|-----------------|-------------|-------------|-------------|
| Control (P1)    | 50.00±4.67  | 59.50±12.79 | 23.50±1.36  |
| Serum 10% (P2)  | 54.3±3.70   | 64.60±7.79  | 21.00±1.36  |
| Serum 20% (P3)  | 66.00±9.45  | 75.20±2.41  | 14.20±1.30  |

3.3. Motility percentage
The motility of cauda epididymis spermatozoa of Swamp Buffalo on TALP media with 0%, 10% and 20% serum treatment after 4 hour preservation at 5°C was 50.00±4.67, 54.3±3.70, 66.00±9.45. Based on these data it can be seen that the TALP media with 20% serum treatment after 4 hours preservation can increase the motility of cauda epididymis spermatozoa of Swamp Buffalo and significantly different (P<0.05) from 0% and 10% serum treatment. The result of this research was higher than the result of [16] and [3]. [4] on cauda epididymis spermatozoa of African Buffalo (syncerus caffer) that are 60±3,82% and 33±12,51%. This difference was tought to be due to differences in Buffalo species and individual condition of buffalo that used in the research. TALP medium with addition serum treatment made motility percentages high because serum treatment effectivey maintained the viability of spermatozoa. The serum contains a lot of nutritious component that very useful to maintain viability when liquiding progress. According to [17] the addition of serum in the spermatozoa dilucent with egg yolk can stimulate metabolism of spermatozoa in a short time and therefore can be used for a short time when diluenting cow spermatozoa. Equilibration time is the time of self adjustment of spermatozoa with diluent so by that when spermatozoa been freezing it can minimalize the damages. The different in equilibration time will affect sperm with different motility result after freezing. Equilibration time in optimum condition will increase spermatozoa motility because it will give glicerol best condition to protect spermatozoa from cold shock during freezing proces. Glicerol will penetrates into the spermatozoa cell to creat an equal intracellular and extracellular concentration. In equilibration there is not only created balance of glycerol concentration but also other active extender osmotic compound [18].

3.4. Viability Percentages
The viability of cauda epididymis spermatozoa of Swamp Buffalo in TALP Medium after 5 hours equilibration at 5°C with 0%, 10% and 20% serum treatment was 59.50±12.79, 64.60±7.79 and 75.20±2.41. TALP medium with addition 20% serum treatment gave the highest result of cauda epididymis spermatozoa viability of Swamp Buffalo after preservation significantly different (P<0.05) with 0% and 10% serum treatment. Based on those data, it could be seen that serum treatment can be an alternative of good profile amino acid as protein source that protect spermatozoa. [8] Stated that blood plasma contain several chemical compound such as water, gas, protein, glucose, fat, non protein substance, nitrogen, enzyme, hormon, vitamin and pigmen that very suit with spermatozoa condition. Protein plasma consist of 90% water and 10% solid. These solid consist of 7% various protein and 0.9
organic material and the other is an organic non protein material. Solid organic comprise 7% protein that include antibody, Phospolipid, cholesterol, glucose, enzyme and hormone. Meanwhile anorganic non protein material comprices Na, C, k, Mg, P, J, Fe, Cu and HCO3. The compotition of plasma and animal blood serum is very complicated. Furthermore one of the hormon in animal blood serum contain growth hormone.

The different of equilibration time also create different result of spermatozoa viability after freezing. In maintaining spermatozoa cell condition, gliserol takes sufficient time to enter the cell membrane and protect the organel cell from freezing damages (cold shock). With Optimum equilibration time, diluent is able to work optimally to prevent ice crystalization that damage spermatozoa. By adding glycerol that bind to water molecules so it create small and soft ice crystal of ice that is not dangerous for spermatozoa cell. When the damage of membran organel cell can be avoided, so by that the function of each cell goes normally. Also then 4 hour equilibration time will provide enough time to create balance between intracellular and extracellular.

3.5. Abnormality spermatozoa percentages
The percentages of abnormal cauda epididymis spermatozoa of Swamp Buffalo in TALP medium with 0%, 10%, and 20% serum treatment is 23.50±1.36, 21.00±1.36 and 14.20±1.30. The best result with the lowest rate of abnormal cauda epididyis spermatozoa of Swamp Buffalo in this research was 14.20±1.30 with 20% serum treatment and significantly different (P < 0.05) from treatment 0% and 10%. The result of this research about abnormal spermatozoa is still in normal limit for artificial insemination. Similar to research done by [12] and [19] that as long as abnormal spermatozoa has not reached 20% then the spermatozoa can be used for artificial insemination program. According to [20], cement usually contain 5% abnormal spermatozoa, fertility will not be disturbed to an abnormal spermatozoa until rate 20-25%. Abnormal spermatozoa do not show progressive move. Abnormal spermatozoa are usually caued by cold shock or overheated temperature, x-ray, nutrition imbalance and endocryne system [21].

The abnormal form of cauda epididymis spermatozoa in these research are mostly secondary abnormality were found such as rolled tail, broken neck, head and tail. Secondary abnormality also found when preparatory preperation [22]. In addition, form of primary abnormalities such as the head is to small (microcheapalic) and double tail. [23] mentioned that the longer storage time, it will cost abnormality rate increasing caused by cold shock and imbalance osmotic pressure from metabolic processes during storage at 5oC temperature.

4. Conclusion
Based on the result of the research it can be concluded that TALP medium with 20% serum addition can maintain the motility, viability and decreasing abnormality of cauda epididymis spermatozoa of Swamp Buffalo.

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