Prosiding_IOP_The Preservation_Harissatria_2021

by Harissatria Harissatria

Submission date: 13-Jun-2023 04:59PM (UTC+0800)
Submission ID: 2115131485
File name: oa_of_Buffalo_into_Talp_Diluent_with_Addition_of_Serum_Level.pdf (781.34K)
Word count: 3393
Character count: 18540

The Preservation Epididymis Spermatozoa of Buffalo into Talp Diluent with Addition of Serum Level

The Preservation Epididymis Spermatozoa of Buffalo into Talp Diluent with Addition of Serum Level

Harissatria[1], Dara Surtina[1], Jaswandi[2], Hendri[2], T Astuti[1], D Afrini[3], R M Sari[1], J Hendri[1], A Asri[1]

[1]Department of Animal Science, Faculty of Agriculture University of Mahaputra Muhammad Yamin, Jalan Jenderal Sudirman No 6 Solok City, West Sumatera. Indonesia. . E-mail: haris_satria85@yahoo.com
[2]Department of Animal husbandry, Faculty of Animal husbandry, Andalas University, Jalan Limau manis, Padang. 25166. West Sumatera. Indonesia
[3]Department of Agribusiness, Faculty of Agriculture University of Mahaputra Muhammad Yamin, Jalan Jenderal Sudirman No 6 Solok City, West Sumatera. Indonesia

Abstract. The purpose of this study is to determine the quality of epididymis spermatozoa of buffalo after preservation with TALP media by adding serum with different level. The method used in this study is the experimental method and the design used is a randomized block design (RBD) with 3 treatment by adding bovine serum (0%, 5%, and 10%) and there are 6 replications as a group (repetition in the form of days). The result showed that the addition of serum into TALP diluent had a significant effect (P>0, 05) on the percentage of the motility of cauda epididymis spermatozoa of swamp buffalo after preservation which was 73, 33 in P2 10% serum and the viability is 79,167 ± 2,04 on P2 10%. This can be used as an indicator that the serum is effective in protecting spermatozoa from damages during the cryopreservation process, so by that, it would improve the quality of cauda epididymal spermatozoa of swamp buffalo. While the percentages of abnormality of cauda epididymis spermatozoa of swamp buffalo after preservation did not have a significant effect (P>0, 05). Based on the result of this study it can be concluded that the addition of 5% serum and 10% addition of egg yolk and 5% addition of glycerol will improve the quality of motility and viability and had no adverse effect on abnormalities after the preservation process at low temperature.

1. Introduction

The effort made to avoid the loss of genetic material from many of swamp buffalo are slaughtered in abattoirs is to save genetic material from these animals, so it can be used again through the technological application. Rescue of genetic material for the preservation of germplasm from dead or cut livestock can be done with the help of reproductive techniques that have developed a lot, including in male animal with the use of spermatozoa originating from the epididymis [1]; [2]. Spermatozoa that came from cauda epididymis have the ability to fertilize oocyte as well as ejaculated spermatozoa [3].

According to Ref. [4] in the process of cement preservation at low temperature (generally at temperature 3-5°C and -196°C) will damage the spermatozoa because of the effect of cold shock that can damage the membrane of the cell plasma and result in the death of spermatozoa. To minimalize the damage to the membrane of the cell plasma because of the cold shock, the effort that can be done is by add certain substances into cement diluents [5]. These substances are known as cryoprotectants such as

glycerol (intracellular cryoprotectant) and other types of sugar (extracellular cryoprotectants) [6] that can be used during cryopreservation process and cement preservation at 3-5°C.

In the process of cryopreservation of cement, due to the low-temperature treatment (-196°C) will crystallization the cement and change the concentration of intracellular electrolytes that cause damages to spermatozoa cell. Choosing the right type of diluent is an alternative way to decrease damage due to the freezing process. Diluent that has been commonly used in the cryopreservation process of cement and is widely known are skim milk diluents, fresh milk, tris, citrate, lactose but the of TALP (Tyrode Albumin Sodium Lactate Sodium Pyruvate) has never been tested on swamp buffalo cauda epididymis spermatozoa as diluent but it is often used at in vitro fertilization media.

This TALP medium has a complete chemical composition such as NaCl, KCL, CaCL, MgCL, NaH_2PO_4, $NaHCO_3$, Hepes, Pyruvate Acyd, Gentamicin, Caffeine . While serum function can improve the quality of spermatozoa as stated by Ref. [7] plasma or blood serum contains a lot of glucose, fat, non-protein substance, nitrogen, enzymes, hormones, vitamins and pigments. The plasma protein consists of 90% water and 10% solid. This solid material consists of 7% protein which includes antibodies, phospholipid-cholesterol, glucose , enzymes while inorganic ingredients, not proteins consist of P, Na, Ca, K, Mg, Fe, and HCO_3. With the advantage of TALP media for spermatozoa into the fertilization process, it is necessary to combine the TALP media with the addition of serum.

The solution that can improve the quality of spermatozoa is by use TALP diluents media with the addition of fresh milk serum at the time of estrus. The advantage that can be obtained from this TALP medium is because it has a complete chemical composition such as NaCl, KCL, CaCL, MgCL, NaH_2PO_4, $NaHCO_3$, Hepes, Pyruvate acid, Gentamicin, Caffeine. While serum function can improve the quality of spermatozoa as stated by Ref. [7] plasma or blood serum contains a lot of glucose, fat, non-protein substance, nitrogen, enzymes, hormones, vitamins and pigments. The plasma protein consists of 90% water and 10% solid. This solid material consists of 7% protein which includes antibodies, phospholipid-cholesterol, glucose, enzymes while inorganic ingredients, not proteins consist of P, Na, Ca, K, Mg, Fe, and HCO_3.

The addition of serum in the diluent of spermatozoa cauda buffalo epididmis can improve the quality of life percentage and reduce spermatozoa abnormalities [8]. Based on this description, the purpose of this study was to determine the quality of buffalo epididymal cauda spermatozoa in TALP diluents after preservation at 5 °C.

2. Material and Method

Testicles with epididymis of swamp buffalo were taken from abattoirs in the Padang City. The epididymis is separated from the testis then cleaned and put into a glass tube that has been filled with a physiological NaCl solution and taken to the laboratory under ambient temperature conditions. After arriving in the laboratory the scrotal skin and tunica vaginalis were incised and the testicle was handled as aseptically as possible after removal, spermatozoa will be collected from the cauda epididymis by making incisions using sterile scissors then rinsing them with a 4 ml physiological NaCl solution [9]).

The collection of spermatozoa was divided into four test tubes of the same volume, then centrifuged at 3000 rpm for 30 minutes. Discarded supernatants and sediment (spermatozoa) were diluted again with TALP diluents at a concentration of 100 million motile spermatozoa per 0,25 ml. The diluents treatments tested were: P1 which contains TALP diluents containing 20% egg yolk, 5% glycerol and 0% serum. P2 which contains TALP diluents containing 20% egg yolk, 5% glycerol and 5% serum. P3 which contains 10% egg yolks, 5% glycerol and 10% serum.

Diluted spermatozoa are packaged in a mini straw (0,25 ml) and then calibrated in the refrigerator at 3-50C for three hours. After equilibration, the spermatozoa is frozen by placing the straw 10 cm above the surface of liquid nitrogen temperature around – 130°C) for 15 minutes. Then the straw is put into the liquid nitrogen container (temperature around -196°C). After being stored for 1 hour, each straw treatment in thawing by entering into 37°C temperature water in a water bath for 30 seconds to evaluate the quality of spermatozoa.

2.1. Evaluation of the quality spermatozoa microscopic

The quality of spermatozoa evaluated after treatment was the percentage of motile spermatozoa, live spermatozoa, and abnormalities. The percentage of motile spermatozoa is the percentage of progressive moving spermatozoa, determined subjectively at eight different visual fields with a light microscope magnifying 400 x. The number is given a range between 0% and 100% with a 5% scale. The percentage of living spermatozoa is determined by using 2% eosin B coloring [10]. Living spermatozoa are marked by a white head, while the death spermatozoa are marked by a redhead. At least 200 of spermatozoa were evaluated by a light microscope with 400x magnification. The percentage of abnormal spermatozoa was evaluated by preparations used to evaluate the percentage of life spermatozoa.

2.2. Analysis of Data

Data were analyzed by analysis of variance in the form of a randomized block design with three treatments and six replications as a group (sampling day). The difference between treatments was tested with the smallest real difference test [11].

3. Result and Discussion

The quality of epididymal cauda spermatozoa which includes the percentage of motility, life percentage and percentage of abnormalities can be seen in Table 1.

Table 1. Quality of buffalo epididymic cauda spermatozoa in serum addition treatment after equilibration

Parameter	Serum Treatment (%)		
	0	**5**	**10**
Motility (%)	68.67±2.94[a]	70.33±0.81[b]	73.33±2.58[b]
Viability (%)	74.167±2.04[a]	77.333±1.96[b]	79.167±2.04[b]
Abnormality (%)	19.33±1.03	19.03±1.06	19.00±1.41

The results showed that adding a serum to TALP diluents had a significant effect (P>0,05) both on the percentage of motility and viability from epididymis spermatozoa of swamp buffalo after cryopreservation. This can be used as an indicator that the serum has effectively protecting spermatozoa from damages during the cryopreservation process so that it can improve the quality of epididymis spermatozoa from swamp buffalo. The result of motility and viability from this research is higher from Haris *et al* research [8] that the motility is 51,80±6,90% and the viability is 64.60±5.54%. The increasing of motility and viability percentage on this research caused by adding serum into TALP media that can use as protein resource in diluent media by the spermatozoa, The serum has various essential components such as protein, hormones, growth factors, minerals and lipids [12];[13]. Many studies state that serum plays an important role in fertilization media in vitro. The supplementation serum in maturation media aims to increase the maturation rate. Various types of serum can be used as supplementation material in maturation media of sheep a goat oocytes liked; fetal bovine serum (FBS) [14];[15], estrus sheep serum (EES) [15]; [16]. Sheep serum (SE) [17], Pregnant Sheep Serum, (PSS) [16], fetal serum calf (FCS) [18];[19] and serum estrus goat(EGS) [20].

The composition or ingredients contained in the serum very depends on the time at which the blood sample is taken for its research, wethers it is not estrus, estrus or pregnant. The serum used in this research is taken during estrus will give a good response because serum contains hormones and other potential growth factors [21].

Furthermore, the high percentages of motility and viability in this research were caused by the addition of 20% egg yolk and 5% glycerol in TALP media. With the presence of egg yolk in diluent media as much as 20% will help spermatozoa to get food and energy sources to maintain motility and viability. Then, with the addition of 5% glycerol, it will able to protect the spermatozoa from cold shock during cryopreservation and as cryoprotectant intracellular.

This result same with the opinion of Ref. [22] that the addition of glycerol until 6% into Tris diluent is able to provide protection against damages for cement. Furthemore Ref. [22], the effect Tris

diluent is to maintaining the balance of intracellular and extracellular electrolytes so by that the biochemical processes that in spermatozoa cells continue and reduce excessive spermatozoa cell death. One of the adverse effects is cold shock where the effect is the death of spermatozoa after thawing due to the high contraction from cell wall lipoprotein.

Glycerol has another benefit which is to prevent dehydration because it has a strong water binding capacity [10]. This will affect the vapor pressure so that the freezing point of the medium decreases, consequently spermatozoa cells will have a longer chance to release water. Glycerol will provide affective protection towards spermatozoa during the freezing process if the concentration in diluent optimal.

The result of this research about the percentage of abnormality spermatozoa of cauda epididymis from swamp buffalo after preservation did not have a significant effect (P>0, 05). The abnormalities that found in cauda epididymis spermatozoa from swamp buffalo on this research are still in the normal range and can be used for the next procedure because according to Ref. [23] the standard rate for abnormal morphology is 10 until 25% will not give bad impact to fertility. Abnormalities can be influenced by two factors, first from primary factors like age, condition of the body of the livestock and the type of livestock itself, while secondary factor is related to the handling of the cement and the technical error in the laboratory. Ref. [24] stated that the abnormality caused by secondary factors are more in the form of the tail separation from the head due to the disconnection during preparation for evaluation purpose. Ref. [25] state that lipid peroxidation will create structural damages that disturb spermatozoa metabolism which results in dead spermatozoa.

Ref. [23] mentioned that if abnormalities more than 25% from one ejaculated spermatozoa, fertility reduction cannot be anticipated. Mature spermatozoa inside epididymis and cytoplasmic droplet move from proximal droplet to distal droplet and finally disappears before ejaculation [26]. According to Ref. [26], primer abnormality have characteristic liked ahead to large (macrocephalic), the head too small (microcephalic), a short head, flat, double head, double tail, bending, enlarging, piriformis or abaxial linking at the base of the head and circular, broken or split tail which is a genetic factor of each animal. Meanwhile, secondary abnormality includes broken tails, a head without tails, the middle section that folds as a result of treatment during the collection process and a factor in human error. Every abnormal spermatozoon cannot fertilize the ovum, regardless of whether the abnormality occurs in the seminiferous tubules in the epididymis. As long as the percentage of abnormal spermatozoa below 20% of the cement sample, then the cement can still be used for insemination [10].

4. Conclusion

Based on the result of this research it can be concluded that the addition of 5% serum and 10% serum into TALP diluent with the addition of 20% of egg yolk and 5% glycerol will improve the quality of motility and viability but does not have a significant effect on abnormalities after the preservation process at low temperature.

References

[1] Hori, T., M. Ichikawa, E. Kawakami and T. Tsutsui. 2004. Artificial insemination with frozen epididymal sperm beagle dogs. J.Vet. Med.Sci 66 (1) : 37-41.

[2] Nazlie, C.S. 2004. Kajian kualitas spermatozoa kucing asal epididimis dan ductus deferens setelah C. proses preservasi selama 7 hari pada suhu 4 Thesis. Sekolah Pascasarjana. Institut Pertanian Bogor. Bogor.

[3] Hafez, E.S.E. and B. Hafez. 2000. Reproduction in Far Animals. 7th Ed. Baltimore USA: Lippincott Williams & Wilkins.

[4] White. I. G. 1993. Lipid and calcium uptake of sperm in relation to cold shock and preservation : A review. Reprod Fertil Dev 5: 639-658.

[5] Kayser. J.P, Amann. R. P, Shidefer. R. K, Squires. E. L, Jasko. D. J, Pickett. B. W. 1992. Effects of linear cooling rate on motion characteristics of stallion spermatozoa. Theriogenology 30: 601-614.

[6] Supriatna, I. dan F.H. Pasaribu. 1992. In Vitro Fertilisasi, Transfer Embrio dan Pembekuan Embrio. Pusat Antar Universitas, Institut Pertanian Bogor, Bogor.

[7] Ganong W.F. 2003. Buku Ajar Fisiologi Kedokteran.Widjajakusumah HMD Penerjemah; Jakarta: Penerbit Buku Kedokteran EGC. Terjemahan dari: Review of Medical Physiology.
[8] Harissatria., D. Surtina., T. Astuti., Jaswandi and Hendri. 2019. Viability Spermatozoa Epididymis of Buffalo (Bubalus Bubalis) In Fertilized Media to Additional Serum at Temperature 5°C. 6th International Conference on Sustainable Agriculture, Food and Energy. IOP Conf. Series: Earth and Environmental Science 347.
[9] Rizal, M. 2004. Penyimpanan epididimis domba pada suhu 5°C selama tiga hari: Pengaruhnya terhadap kualitas spermatozoa yang telah dibekukan. Media Kedokteran Hewan. 20:57-61.
[10] Toelihere,M.R. 1993. Inseminasi Buatan pada Ternak. Penerbit Angkasa, cetakan ke-3, Bandung.
[11] Steel R.G.D, Torrie J.H. 1993. Prinsip dan Prosedur Staistika. Gramedia Pustaka Utama, Jakarta.
[12] Langendonckt, A.V., Donnay, I., Schuurbiers, N., Auquier, P., Carolan, C., Massip, A & F. Dessy. 1997. Effects of suplementation with fetal calf serum on development of bovine embryos in synthetic oviduct fluid medium. J. Reprod. and Fertil. 109: 87-93.
[13] Boediono A, Rusiyantono Y, Mohamad K, Djuwita I, Herliatien. 2000. Perkembangan oosit kambing setelah maturasi, fertilisasi dan kultur in vitro. Media Vet 7: 11-17.
[14] Wani, N.A., Wani, G.M., Khan, M.Z & S. Salahudin. 2000. Effect of oocyte harvesting technique on in vitro maturation and in vitro fertilization in sheep. Small Rumin. 36: 63-67.
[15] Nava, G.H & H. Tajik. 2000. In vitro maturation of ovine follicular oocytes in different concentrations of fetal calf serum and estrous sheep serum. Theriogenology 53: 435.
[16] Wattimena, J. 2006. Pengaruh serum domba estrus dan serum domba bunting terhadap produksi embrio domba in vitro. Jurnal Ilmu Ternak dan Veteriner. 11: 116-122.
[17] Wattimena, J. 2004. Pengaruh Suplementasi Jenis dan Konsentrasi Serum Terhadap Produksi Embrio Domba Secara In Vitro. [Disertasi]. Bandung: Univesitas Padjadjaran.
[18] Rho, G.J., Hahnel, A.C & K.J. Betteridge. 2001. Comparisons of Oocytes Maturation Times and of Three Methods of Sperm Preparation for Their Effects on The Production of Goat Embryos In vitro. Theriogenology 56: 503-516.
[19] Wang, Z.G., Xu, Z.R & S.D. Yu. 2007. Effetcs of oocytes collection techniques and maturation media on in vitro maturation and subsequent embryo development in boer goat. Czech J. Anim. Sci. 52: 21-25.
[20] Rahman, A.N.M.A., Abdullah, R.B & W. Khadijah. 2007. Goat Embryo Development From In vitro Matured Oocytes of Heterogenous Quality Trough Intracyto-plasmic Sperm Injection Techniques. Biotechnology 6: 373-382.
[21] Younis, A.I., Brackett, B.G & R.A.F. Hosken. 1989. Influence of serum and hormone on bovine oocytes maturation and fertilization in vitro. Gamete Research, 23: 189-201.
[22] Tambing, S.N., M.R. Toelihere, T.L. Yusuf, & I.K. Sutama. 2000. Pengaruh gliserol dalam pengencer Tris terhadapkualitas semen beku kambing Peranakan Etawah. JITV5:1-8.
[23] Bearden, H.J. and J.W. Fuquay. 1997. Applied Animal Reproduction, 4thed. Prentice Hall. New York.
[24] Rizal M. 2006. Fertilitas semen beku hasil ejakulasi dan spermatozoa beku asal cauda epididimis domba garut. J Sain Veteriner 24: 49-57.
[25] Alawiyah, D., dan Hartono, M. 2006. Pengaruh Penambahan Vitamin E dalam Bahan Pengencer Sitrat Kuning Telur terhadap Kualitas Semen Beku Kambing Boer. Journal. Indonesia. Tropical. Animal. Agriculture. 31[1] March 2006.
[26] Toelihere.M.R. 1985.Fisiologi Reproduksipada Ternak.Penerbit Angkasa, Bandung.

Acknowledgments

The author would like to thank to the head if livestock biotechnology laboratory of the Faculty of Animal Husbandry of Andalas University because it has helped author in facilitating tool and material during the research activity, as well at research members who have helped in writing this article.

Prosiding_IOP_The Preservation_Harissatria_2021

ORIGINALITY REPORT

16%	16%	6%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

9%

★ repository.lppm.unila.ac.id

Internet Source

Exclude quotes	On	Exclude matches	< 3%
Exclude bibliography	Off		