Scholars Journal of Agriculture and Veterinary Sciences OPEN ACCESS
Abbreviated Key Title: Sch J Agric Vet Sci
ISSN 2348-8883 (Print) | ISSN 2348-1854 (Online)
Journal homepage: www.saspublishers.com

Fertility of Buffalo Cauda Epididymal Sperm with Swim up Method

Harissatria[1*], D. Surtina[1], Jaswandi[2], Hendri[2] and Rizqan[3]

[1]Department of Animal Science, Faculty of Agriculture, Mahaputra Muhammad Yamin University, Solok, West Sumatera (27317) Indonesia
[2]Departement of Animal Science, Faculty of Animal Science, Andalas University West Sumatera 25163, Indonesia
[3]Postgraduate Program of Animal Science, Faculty of Animal Science, Andalas University West Sumatera 25163, Indonesia

***Corresponding author:** Harissatria
| **Received:** 02.06.2019 | **Accepted:** 11.06.2019 | **Published:** 19.06.2019
DOI: 10.36347/sjavs.2019.v06i06.001

Abstract **Original Research Article**

This study was conducted to evaluate the use of swim up methods in vitro fertilization on buffalo oocytes. Buffalo epididymis cauda was sexed with 3 sexing treatments P1 (1 hour), P2 (2 hours) and P3 (3 hours) in Co_2 incubator 5%. Oocyte fertilized in vitro with Cauda Epididymal Sperm in each treatment. The results showed that the fertilization rate of cauda epididymal sperm after swim up showed very significant differences ($P < 0.01$) in P2 treatment with a 2-hour swim-up incubation duration in Co_2 incubator 5% with a value of 95,222 ± 3.71%. 2 cell division rates showed very significant differences ($P < 0.01$) in P2 treatment with a value of 88.97 ± 5.54 and not significantly different in the development of 8 cells ($P > 0.05$). It can be concluded that the swim up method has an effect on the level of fertilization and development of oocytes to 2 cells and there has been a decrease in development at the level of 8 cells in buffalo oocytes in vitro.

Keywords: buffalo, in-vitro fertilization, cauda epididymal, swim up.

Copyright © 2019: This is an open-access article distributed under the terms of the Creative Commons Attribution license which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use (NonCommercial, or CC-BY-NC) provided the original author and source are credited.

INTRODUCTION

Rescue genetic material from male buffalo cattle that have died or cut can be done with the use of spermatozoa collected from the cauda epididymal these animals [1]. Collection and cryopreservation of spermatozoa from the cauda epididymis may be one way of saving genetic material from animals that have died. Cauda epididymal spermatozoa collection origin from a slaughterhouse is a quick and inexpensive alternative and cauda epdidymal have live sperm count high enough [2].

Cauda epididymal sperm motility, plasma membrane integrity are not different from ejaculated spermatozoa from either before or after dikriopreservasi [3] and still have the ability to fertilize oocytes in vitro and produce offspring [4, 5]. The cauda epididymal spermatozoa are competent of fertilizing egg cells in vitro as is done with red deer [6] and horses [7]. In vitro fertilization ability of cauda epididymal spermatozoa after storage cryopreservation such as cows [8] which showed its ability to produce embryos in vitro using sperm collected from the cauda epididymal and stored at 5°C. [9] Blash *et al.* reported in goats, cryopreservation of spermatozoa collected from the cauda epididymis were able to fertilize oocytes in vitro by 40% with the cleavage and 6% developed into blastocysts.

Motility of spermatozoa is an important parameter in the fertilization ability of sperm and increase the ability of sperm to penetrate the pellucida zone of the oocyte [10]. Accordingly a method for separating living spermatozoa is needed to increase the percentage of motile spermatozoa. Various methods of spermatozoa selection were carried out to improve the motility of spermatozoa in in vitro fertilization, one of which was by using the swim up method. Swim up is a cheap and easy method to implement [11] where this method selects high motility spermatozoa that can reach the media surface after incubation [12]. Swim up method is done to separate motile spermatozoa from which no motile spermatozoa or die, and initiated capacitation [13]. In sheep [14] reported the swim up method was able to select live spermatozoa and those who did not experience apoptosis. Based on this, the swim up method was carried out in the process of preparing spermatozoa before fertilization by cutting spermatozoa from the cauda epididymal of swamp buffalo. The application of the swim up method is expected to be one method to improve the ability of in vitro fertilization of spermatozoa from swamp buffalo cauda epididymal.

RESEARCH MATERIALS AND METHOD

Oocyte collection and maturation in vitro on the first day

Ovaries of buffalo livestock are obtained from slaughterhouses. Oocytes are collected by slicing the ovary cortex using a micro blade. Oocyte collection was carried out using a Phosphate Buffer Solution (PBS) and supplemented with 0.3% of serum which was first calibrated in a 5% Co_2 incubator. The oocytes used are oocytes with homogeneous cytoplasm and have more than a few layers of compact cumulus cells. Furthermore oocytes are matured in TCM-199 media (Sigma, USA) supplemented with 0.3% serum, 10 µg / mL Follicle Stimulating Hormone (FSH; Teikokuzoki, Tokyo, Japan), and 50 µg / mL gentamycin (Sigma-Aldrich. St. Louis, MO, USA). Oocyte maturation was carried out in a maturation medium in the form of a drop of 100 µL for 10-15 oocytes and protected with mineral oil (Sigma-Aldrich. St. Louis, MO, USA) in a Co2 incubator of 5%, at a temperature of 38-39 ° C for 24 hours.

Collection and processing of spermatozoa on the second day

Testicles and epididymis of swamp buffalo were obtained from slaughterhouses (RPH) in the city of Padang. The testicles and epididymis were collected at the time of buffalo slaughter at around 04.00 in the morning, and then transported to the laboratory, Andalas University. Then the spermatozoa are collected around 7:00 a.m. The epididymis is separated from the testis and rinsed with physiological NaCl solution (0.9% NaCl). Spermatozoa are collected by a combination of slicing, rinsing, and pressing (rinse-press) techniques on each tissue of the cauda epididymis [15] using physiological Na Cl solution. Before rinsing-press with physiological NaCl solution, spermatozoa are aspirated by an erythrocyte pipette to calculate its concentration.

Collected spermatozoa are divided into three test tubes with the same volume and concentration. Spermatozoa is extender with: 80% Tris base extender + 20% egg yolk The composition of the Tris base extender consists of: 3.87 g Tris (hydroxymethyl) aminomethane, 2.17 g citric acid, and 1.56 g fructose dissolved with aquabestilate until it reaches volume of 100 ml, then penicillin and streptomycin were added as much as 1,000 IU per milliliter of extender. Spermatozoa are diluted to reach a concentration of 15 million motile spermatozoa per milliliter. Furthermore sealed test tube is then inserted into a beaker containing water and put in a 5% Co_2 incubator with a temperature of around 38-39°C as a treatment that is 1 hour of control treatment, 2 hours of second treatment and 3 hours of third treatment. The purpose of the incubation treatment at 38-39°C was to obtain motile spermatozoa from the swim-up process.

In vitro fertilization

Preparation of spermatozoa for fertilization is carried out by swim up according to the treatment for a long swim-up time of 1 hour, 2 hours and 3 hours. Spermatozoa which have been carried out by the swim up process are taken 100 µl of the upper fraction using micropippets and are inserted into the TALP media (Tyrode Albumin Lactate Pyruvate) which is supplemented with glucose and serum. Matured oocytes are inserted into spermatozoa drop and incubated for 12-14 hours in CO_2 incubator (5%) on temperature (39°C).

Variables observed

- The percentage of fertilized buffalo oocytes is indicated by the emergence of the 2nd polar body
- Percentage of oocyte development to 2 cells
- Percentage of oocyte development to 8 cells

Data analysis

The design used in this study was Randomized Block Design (RBD) with 3 treatments and 6 replications as a group (days). Data were analyzed statistically using analysis of variance (Analysis of Variance / ANOVA) [16].

RESULT AND DISCUSSION

Percentage of fertilized using the sperm swim-up methods

Evaluation of the growth oocyte nucleus after in vitro fertilization is done by preparation of pronucleus (PN) formation, and fertilized oocytes are characterized by the formation of two pronucleus (2PN). The level of buffalo oocyte fertilization using cauda epididymal sperm that received swim-up P1 (1 hour), P2 (2 hours) and P3 (3 hours) treatment showed the results of fertilized percentage as shown in Table 1.

Table-1: Percentage of oocytes fertilized using the sperm swim-up methods

Treatment	Mean and Standard Deviation
P1	84.550 ± 2.36^{a}
P2	95.222 ± 3.71^{b}
P3	92.998 ± 4.23^{c}

Note: different superscrib of abc shows significance difference (P<0.01)

The results of the study in Table 1 show that spermatozoa originating from the cauda epididymal with swim-up treatment before fertilization showed a very significant difference (P <0.01) in treatment P2

with a 2-hour swim-up incubation duration in 5% co_2 incubator with a value of 95.222 ± 3.71. Fertilization rates in this study were supported by the results of a study by Harissatria and Hendri [17], which was 88.98% in fertilizing buffalo oocytes. This is because the 2-hour swim-up is a good time for spermatozoa to carry out the process of capacitation and maturation while separating motile spermatozoa or do not. The more mature the spermatozoa, the higher the ability of the spermatozoa to fertilize the oocyte.

At 2 hours the swim-up of spermatozoa is good for selecting motile spermatozoa and is able to fertilize the oocytes. Swim up method is one of the techniques of separation of spermatozoa based on the movement of motile spermatozoa towards the surface of the media after incubation. Mechanical separation of semen plasma or cryoprotectant and separation of motile spermatozoa that are not motile can be done to improve the ability of spermatozoa in vitro fertilization [11].

From the results of the study, it was shown that in vitro the various treatments were good because they were able to fertilize oocytes more than 80% and indicated that spermatozoa from the cauda epididymal originated good enough to fertilize the oocytes in vitro. This is consistent with the statement [3,18] that spermatozoa originating from the cauda epididymal have motility, plasma membrane integrity and morphology that are not different spermatozoa from ejaculation. Spermatozoa from the cauda epididymal have the ability to fertilize oocytes as well as spermatozoa from ejaculate [19]. This is because the spermatozoa found in the cauda section have passed the ripening process in the head and corpus epididymal and already have the same motility as spermatozoa from ejaculate [20].

Percentage of oocyte development to 2 cells

Evaluation of oocyte development to 2 cell after in vitro fertilization is done by observing cytoplasmic division into two parts. The level of division of buffalo oocytes to two cells using cauda epididymal spermatozoa that received swim-up P1 (1 hour), P2 (2 hours) and P3 (3 hours) treatment showed the percentage percentage results as shown in Table 2.

Table-2: Percentage of oocyte development to 2 cells using the sperm swim-up methodes

Treatment	Mean and Standard Deviation
P1	70.96 ± 10.78^{a}
P2	88.97 ± 5.54^{b}
P3	84.69 ± 4.69^{c}

Note: different superscrib of abc shows significance difference (P<0.01)

The results of the study in Table 2 show that the oocytes to divide up to 2 cells with swim-up spermatozoa showed very significant differences (P <0.01) in P2 treatment with a value of 88.97 ± 5.54. This indicates that the higher the percentage of oocytes successfully fertilized, the higher the number and level of cell division. The use of appropriate culture media will have a good impact on growth of embryo. This is consistent with the statement of Meirelles *et al.* [21] and Nedambale *et al*. [22] that earlier embryos to growth have greater potential to divide. This is due to the nature inherited by the cytoplasm and correlates with the maternal zygote transition function [21].

In this study the count of embryos splitting more and the 2-hour swim-up treatment time in incubator co_2 (5%) compared to 1 and 3 hours of swim-up. This indicates that the culture of post-fertilization embryos is the most important period to be observed because it determines the speed of cleavage [23, 22] and embryo quality [24].

Percentage of oocyte development to 8 cells

Evaluation of oocyte development to 8 cells after in vitro fertilization is done by improving cytoplasmic division into 8 sections. The level of division of buffalo oocytes to 8 cells using cauda epididymal sperm that received swim-up P1 (1 hour), P2 (2 hours) and P3 (3 hours) treatment showed the percentage percentage results as shown in Table 2.

Table-3: Percentage of oocyte Development to 8 cells using the sperm swim-up methodes

Treatment	Mean and Standard Deviation
P1	20.27±9.89
P2	21.12±4.36
P3	16.84±7.13

The low number of embryo development obtained is caused by several factors, including the quality of different oocytes [25, 26] and the culture system used [23, 22, 27, 28, 21] reported that laboratory averages lost 60-70% of the ability of oocytes successfully fertilized to growth into embryos. This is due to the oocytes used for in vitro embryo production collected from the ovaries which generally come from different individuals, resulting in variations in the ability to growth into further embryos. Accordingly it is necessary to have a more accurate ability of oocyte selection techniques so as to produce good quality

embryos in order to be able to activate genomes to support their development and survival [21].

The success of the growth of fertilized oocytes is determined based on their ability to divide and continue to growth the next stage. At the beginning of embryo development is supported by the availability of mRNA and transcription activities by the maternal before genomic activation begins [25]. Goeseels and Panich state that genome activation in embryos begins at 8-16 cell division while [21] Meirelles *et al*. states that genomic activation begins in stage 8 cells. Elder and Dale [29] add that the synthesis of new proteins as markers of the initiation of genomic activation in the embryo occurs at the division of 4-8 cells. The transition from maternal to embryo is characterized by active transcription by the embryo genome because maternal mRNA and proteins stored in oocytes are degraded [30, 31].

If there is a failure of the process, it can inhibit gene expression so that the embryo is unable to experience further division [32]. During the transition period, the nucleus programs the activation of the transcription process by the genome of the embryo that has previously inactivated. The success of genome activation is characterized by the ability of the embryo to transcribe mRNA and is no longer dependent on maternal genomes [31]. Referring to the description, the results showed that the percentage of embryos that were able to pass the developmental blockade (8 cell division) showed no significant difference (P> 0.05) in three swim-up treatments. The data shows that only about 16-20% of embryos are able to pass the development blockade of the entire embryo that successfully divides so that it is expected to develop to stage 32 cells (morula).

CONCLUSION

Based on the results of this study concluded that sperm swim up sexing method has the ability to support the fertilization and embryo development until 2 cells but no effect on embryo development up to 8 cells. As a suggestion, further research is needed to evaluate the accuracy of the sex of embryos produced in vitro using sperm sexing X and Y.

REFERENCES

1. Kaabi M, Paz P, Alvarez M, Anel E, Boixo JC, Rouissi H, Herraez P, Anel L. Effect of epididymis handling conditions on the quality of ram spermatozoa recovered post-mortem. Theriogenology. 2003 Oct 15;60(7):1249-59.
2. Ehling C, Rath D, Struckmann C, Frenzel A, Schindler L, Niemann H. Utilization of frozen–thawed epididymal ram semen to preserve genetic diversity in Scrapie susceptible sheep breeds. Theriogenology. 2006 Dec 1;66(9):2160-4.
3. Tebet JM, Martins MI, Chirinea VH, Souza FF, Campagnol D, Lopes MD. Cryopreservation effects on domestic cat epididymal versus electroejaculated spermatozoa. Theriogenology. 2006 Oct 1;66(6-7):1629-32.
4. Jishage K, Ueda O, Suzuki H. Fertility of mouse spermatozoa from cauda epididymis preserved in paraffin oil at 4 C. Journal of Mammalian Ova Research. 1997; 14 (1): 45-8.
5. Songsasen N, Tong J, Leibo SP. Birth of live mice derived by in vitro fertilization with spermatozoa retrieved up to twenty-four hours after death. Journal of Experimental Zoology. 1998 Feb 1;280(2):189-96.
6. Soler AJ, Pérez-guzmán MD, Garde JJ. Storage of red deer epididymides for four days at 5 C: effects on sperm motility, viability, and morphological integrity. Journal of Experimental Zoology Part A: Comparative Experimental Biology. 2003 Feb 1;295(2):188-99.
7. Vieira LA, Gadea J, García-Vázquez FA, Avilés-López K, Matás C. Equine spermatozoa stored in the epididymis for up to 96 h at 4 C can be successfully cryopreserved and maintain their fertilization capacity. Animal reproduction science. 2013 Jan 30;136(4):280-8.
8. Martins CF, Driessen K, Costa PM, Carvalho-Neto JO, De Sousa RV, Rumpf R, Dode MN. Recovery, cryopreservation and fertilization potential of bovine spermatozoa obtained from epididymides stored at 5 C by different periods of time. Animal reproduction science. 2009 Nov 1;116(1-2):50-7.
9. Blash S, Melican D, Gavin W. Cryopreservation of epididymal sperm obtained at necropsy from goats. Theriogenology. 2000 Oct 1;54(6):899-905.
10. Suarez SS, Ho HC. Hyperactivated motility in sperm. Reproduction in domestic animals. 2003 Apr;38(2):119-24.
11. Henkel RR, Schill WB. Sperm preparation for ART. Reproductive biology and endocrinology. 2003 Dec;1(1):108.
12. Wolf CA, Brass KE, Rubin MI, Pozzobon SE, Mozzaquatro FD, De La Corte FD. The effect of sperm selection by Percoll or swim-up on the sex ratio of in vitro produced bovine embryos. Anim Reprod. 2008 Jul;5(3/4):110-5.
13. Centola GM, Herko R, Andolina E, Weisensel S. Comparison of sperm separation methods: effect on recovery, motility, motion parameters, and hyperactivation. Fertility and sterility. 1998 Dec 1;70(6):1173-5.
14. Martí E, Pérez-Pé R, Muiño-Blanco T, Cebrián-Pérez JA. Comparative study of four different sperm washing methods using apoptotic markers in ram spermatozoa. Journal of andrology. 2006 Nov 12;27(6):746-53.
15. Rizal M. Pengaruh penambahan laktosa di dalam pengencer Tris terhadap kualitas semen cair domba Garut. Jurnal Pengembangan Peternakan Tropis. 2006;31:224-31.
16. Steel RG, Torrie JH. Prinsip dan Prosedur Statistika. Suatu Pendekatan Biometrik. Edisi

Kedua. Terjemahan: B. Sumantri. PT. Gramedia Pustaka Utama, Jakarta. 1993.
17. Harissatria H, Hendri J. Fertilitas Dan Persentase Embrio Kerbau Sampai Morula Yang Dikultur Dengan Penambahan Glutathione Secara in Vitro. Jurnal peternakan. 2016 Dec 30;13(1):12-8.
18. Hermansson U, Axnér E. Epididymal and ejaculated cat spermatozoa are resistant to cold shock but egg yolk promotes sperm longevity during cold storage at 4 C. Theriogenology. 2007 Apr 15;67(7):1239-48.
19. Hafez ESE and B Hafez. Anatomy of male reproduction, In: Reproduction in Farm Animals 7th Edition. USA: Lippincott Williams & Wilkins, Baltimore. 2000: 3-12.
20. Axnér E, Holst BS, Linde-Forsberg C. Morphology of spermatozoa in the cauda epididymidis before and after electroejaculation and a comparison with ejaculated spermatozoa in the domestic cat. Theriogenology. 1998 Oct 15;50(6):973-9.
21. Meirelles FV, Caetano AR, Watanabe YF, Ripamonte P, Carambula SF, Merighe GK, Garcia SM. Genome activation and developmental block in bovine embryos. Animal reproduction science. 2004 Jul 1;82:13-20.
22. Nedambale TL, Du F, Yang X, Tian XC. Higher survival rate of vitrified and thawed in vitro produced bovine blastocysts following culture in defined medium supplemented with β-mercaptoethanol. Animal reproduction science. 2006 Jun 1;93(1-2):61-75.
23. Lonergan P, Pedersen HG, Rizos D, Greve T, Thomsen PD, Fair T, Evans A, Boland MP. Effect of the post-fertilization culture environment on the incidence of chromosome aberrations in bovine blastocysts. Biology of reproduction. 2004 Oct 1;71(4):1096-100.
24. Gómez E, Rodríguez A, Muñoz M, Caamaño JN, Hidalgo CO, Morán E, Facal N, Díez C. Serum free embryo culture medium improves in vitro survival of bovine blastocysts to vitrification. Theriogenology. 2008 May 1;69(8):1013-21.
25. Bilodeau-Goeseels S, Panich P. Effects of oocyte quality on development and transcriptional activity in early bovine embryos. Animal Reproduction Science. 2002 Jun 17;71(3-4):143-55.
26. Lonergan P, Fair T. In vitro-produced bovine embryos—Dealing with the warts. Theriogenology. 2008 Jan 1;69(1):17-22.
27. Underwood SL, Bathgate R, Pereira DC, Castro A, Thomson PC, Maxwell WM, Evans G. Embryo production after in vitro fertilization with frozen-thawed, sex-sorted, re-frozen-thawed bull sperm. Theriogenology. 2010 Jan 1;73(1):97-102.
28. Setiadi MA, Karja NW. Tingkat perkembangan awal embrio sapi in vitro menggunakan media tunggal berbahan dasar tissue culture medium (TCM) 199. Jurnal Kedokteran Hewan-Indonesian Journal of Veterinary Sciences. 2013 Sep 1;7(2).
29. Elder K and B Dale. *In Vitro Fertilization*. Ed ke-2. Cambridge University Press. 2003.
30. Gandolfi TB, Gandolfi F. The maternal legacy to the embryo: cytoplasmic components and their effects on early development. Theriogenology. 2001 Apr 1;55(6):1255-76.
31. Graf A, Krebs S, Heininen-Brown M, Zakhartchenko V, Blum H, Wolf E. Genome activation in bovine embryos: review of the literature and new insights from RNA sequencing experiments. Animal reproduction science. 2014 Sep 1;149(1-2):46-58.
32. Betts DH, King WA. Genetic regulation of embryo death and senescence. Theriogenology. 2001 Jan 1;55(1):171-91.