Influence of the transportation of cow ovaries on the IVM/IVF/IVC results: A report

Tsvetelina Todorova, Iancho Todorov*
Department of Reproduction, Institute of Animal Science, Agricultural Academy, 2230 – Kostinbrod, Bulgaria
*Corresponding author: ianchot@abv.bg

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Abstract

Ovaries from 12 cows were collected separately in an abattoir and the data concerning the donor were recorded. The ovaries were transported for 8–10 hours to the laboratory and processed immediately. Oocytes from these ovaries were collected separately and submitted to IVM/IVF/IVC. The seven HF cows supplied 165 oocytes (23.57 per cow on average) with high individual variations. Five MB cows supplied 250 oocytes (50 per cow on average). After IVF 137 expanded blastocysts were obtained (33% of oocytes, 11.41 per cow, range 2–22). All of the obtained blastocysts were frozen by vitrification.

Key words: in vitro, bovine, culture, individual cow

Влияние на транспортирането на яйчници от крави върху резултатите от IVM/IVF/IVC: Доклад

Цветелина Тодорова, Янчо Тодоров*
Институт по животновъдни науки, Селскостопанска академия, 2230 – Костинброд, България
*E-mail: ianchot@abv.bg

Резюме

Яйчниците от 12 крави бяха получени от кланица и поставени по отделно за транспортиране. Данните за донорите бяха записани. Транспортирането на яйчниците до лабораторията беше в рамките на 8–10 часа и незабавно започна обработването им. Получените ооцити бяха събирани поотделно и групирани за IVM/IVF/IVC. От седемте HF крави са получени 165 ооцита (средно 23,57 от крава) с големи индивидуални вариации. От пет MB крави са получени 250 ооцита (средно 50 от крава). След IVF бяха получени 137 експандирани бластоцисти (33% от ооцитите, 11,41 от крава, диапазон 2–22). Всички получени бластоцисти бяха замразени чрез витрификация.

Ключови думи: ин витро, говеда, култивиране, индивидуални крави

Introduction

Initially, the purpose of in vitro fertilization in cows was similar to that in humans: treatment of infertility and obtaining new knowledge on the first moments of life. Some success has been achieved with animals showing pathological infertility, but most of the development and application of the methodology has been aimed at improving genetic selection and reducing the generation interval. Bovine in vitro maturation (IVM), in vitro fertilization (IVF), and in vitro culture (IVC) found some applications in the breeding of high genetic value cattle.

Blastocyst rates in embryos obtained from the in vitro maturation of oocytes and their fertilization and culture are still not at the desired level in comparison to embryos produced in vivo (Diez et al., 2005, Pereira et al., 2005). One of the most important problems encountered in vitro culture studies is seen in the maturation period of oocytes until they reach a fertilizable level. The use of ovaries obtained from animals slaughtered in abattoirs is a practical method employed for the in vitro production of embryos, and also for experimental studies in human medicine (Gardner et al., 2004, Van Soom and Boeijan, 2002).

Transport time, storage of the ovaries, and, in particular, the temperature of the medium used during transport are among the factors affecting complete maturation (Nakao and Nakatsui, 1992).

According to Saleh (2017), that period between slaughtering and sample processing significantly affect oocytes collected percentage and quality. Bacelo-Fimbres et al., 2015, founded that shipping slaughterhouse-derived bovine oocytes either for 18 hours or for 24 hours was superior to the conventional maturation system for blastocyst production at day 7 of IVC.

The development of the ultrasound-guided oocyte aspiration technique in live cows offers the possibility to obtain several oocytes as an alternative method to the current embryo collection procedures involving superovulation. In vitro embryo production offers also an ultimate chance to obtain a surplus offspring from genetically valuable cows after death. Generally, oocytes for in vitro experiments are obtained from ovaries coming from a slaughterhouse and are from animals of unknown origin. Breeding applications of an individual IVM/IVF/IVC method implies the identification of the mother, as well as the father.

In this study influence of the transportation time was tested. In vitro techniques were applied to 12 valuable cows of different breeds that had to be slaughtered because of incurable sterility (6 cows), advanced age (2 cows) and lameness (4 cows).

Materials and Methods

In Vitro Maturation

The ovaries from each particular cow were selected separately in the abattoir. The personal data of each cow was recorded and the ovaries were transported within 8 to 10 hours to the laboratory in 0.9% NaCl at ~ 18–20 °C. Oocytes were recovered by aspiration of all visible follicles regardless of the size and pulled together. After the puncture, the ovaries were sliced using a razor blade in a petri dish filled with MPBS supplemented with 10% FCS and each ovary was washed with fresh MPBS + 10% FCS. The oocytes obtained after slicing were pooled together and because of the danger of mycotic contamination were handled separately from the ones obtained after the puncture. To avoid this the ovaries were handled in the presence of an antibiotic – antimycotic solution (AAS, Sigma). The oocytes were washed two times in MPM (modified Parker's medium) supplemented with 10% ECS and matured in groups of ten in 100 µl drops of MPM supplemented with 20% ECS and 2 µg/ml FSH (Pluset, Calier) for 22–24 hours at 39 °C and 5% CO_2 in humidified air.

In Vitro Fertilization

For the fertilization, frozen semen was used. This was supplied by the owners of the cows. For each breed was used a single bull with approved fertility.

Frozen/thawed sperm was used. Viable spermatozoa were selected using the swim-up meth-

od, washed twice, and diluted to a concentration of ~ 1.10^6 sperms/ml. The matured ova were washed twice in fertilization medium (TALP + 6 mg/ml BSA + 10 µg/ml heparin) and transferred into 45 µl drops of fertilization medium. To each drop 5µl of the sperm suspension was added.

In Vitro Culture

After eighteen hours of sperm/eggs co-incubation the presumptive zygotes were washed twice in MPM without serum and cultured for 24–30 hours in 400 µl MPM without protein supplementation. After this time the cleaved embryos were transferred to 400 µl MPM supplemented with 10 % ECS (not heat-treated) and cultured in the presence of cumulus cells for another 6 days. All of the transferable embryos were frozen by vitrification.

Vitrification

We applied a modified vitrification method described by Do et al., 2016. Details on the composition of the media, the method of preparation, and the procedures are described in detail in an article by Do et al., 2019, Mogas, 2018.

Only blastocysts of the highest quality were vitrified.

Results and Discussion

Our data suggest that transferable embryos could be produced by a complete in vitro procedure. This confirms the results of Mermillod et al., 1992, Van Langendonckt et al., 1995. As mentioned by the authors above, also the yields in terms of oocytes and transferable embryos per cow in our experiments were highly variable.

Also, the yields in terms of oocytes and transferable embryos per cow in our experiments were highly variable. This confirms the results of Maaike et al., 2017. The results are shown in Table 1.

In our experiment in most of the cases, we were able to obtain some more oocytes after slicing the ovaries. Sometimes we recovered more oocytes after slicing compared to aspiration. As mentioned, we cultured these oocytes separately from the oocytes obtained after aspiration because of the danger of mycotic contamination. However, there was no mycotic contamination in all cases studied and the data from aspiration and slicing were pooled together. In Figure 1 a comparison of the development of the embryos between the breeds was made.

Table 1. Development of the oocytes obtained from ovaries of individual cows

Cow No	Breed	Oocytes	> 2 cells		Morula		Blastocyst	
		n	n	%	n	%	n	%
1	HF	22	21	95.45	13	61.90	8	61.53
2	HF	26	25	96.15	13	52.00	10	76.92
3	HF	38	31	81.57	21	67.74	13	61.90
4	HF	15	12	80.00	8	66.66	6	75.00
5	MB	82	61	74.39	25	40.98	16	64.00
6	MB	48	42	87.50	22	52.38	15	68.18
7	HF	36	26	72.22	9	34.61	9	100.00
8	MB	45	40	88.88	25	62.50	22	88.00
9	MB	32	28	87.50	19	67.85	10	52.63
10	HF	18	18	100.00	12	66.66	7	58.33
11	MB	43	37	86.04	24	64.86	19	79.16
12	HF	10	7	70.00	4	57.14	2	50.00

HF – Holstein Frisian, MB – Montbeliard

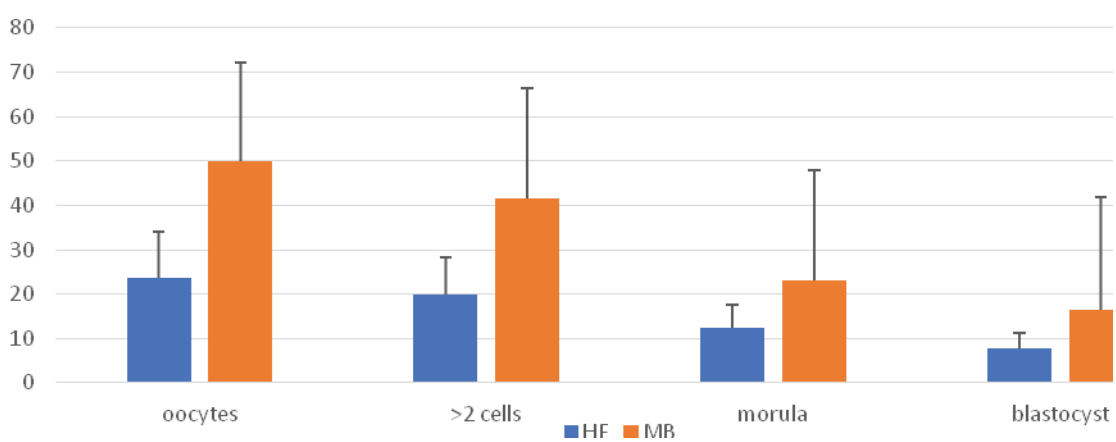
The data shown in Fig. 1 represents the means and SD. The data were compared by unpaired T-test to determine the significant differences. We were unable to find a significant influence of the age of the cows, the reason for slaughtering, and other factors. But we did find significant differences between the breeds. From the figure, one can see there is an influence of the breed. Seven Friesian cows produced fewer oocytes and proportionally fewer blastocysts than five Montbeliard cows. The differences varied from significant ($p < 0.05$) for the oocytes and the zygotes that cleaved to > 2 cells to highly significant ($p < 0.05$) for the development to morula and blastocyst stage.

We have no explanation for those visible differences between the breeds. We will refrain from definite conclusions due to the small number of animals included in the experiment. We can speculate that the Montbeliard cows have a lower milk yield on average than the Holstein cows and this might be one of the possible explanations.

As for the temperature of transportation, Shioya (1993) used to test various temperatures of transportation i.e. 4 °, 23 °, and 38 °C, within 2 to 4 hr. after the death of the animals. The oocytes from the ovaries transported at 4 °C showed a marked decrease of the developmental capability to blastocysts after IVM/IVF. The transportation at 23 °C had an advantage over that at 38 °C, resulting in a development rate of the blastocysts ranging from 15.5% to 20.5%. The possibility of transporting ovaries for a long time was confirmed because 16% of the oocytes obtained from ovaries kept at 20 °C for 8 hr. were able to develop to the blastocyst stage.

Shioya (1993) also reported, that storage of the ovaries for 5–8 hours at a temperature of 37–39 °C decreases the maturation rate of the oocytes and adversely affects their development to the blastocyst stage after in vitro fertilization, Narita et al. (2005). In contrast, storage of ovaries for 8 hours at a temperature of 20–25 °C has been reported to not affect either oocyte maturation rates or development to blastocyst following in vitro fertilization, Abe and Shioya, 1996. Bohlooli et al. (2015) transport the ovaries to the laboratory for about 3 hours, testing three transport medium temperatures of 4 °C, 25 °C, and 38 °C. The best results are obtained at a temperature of 4 °C. These results are very indicative of the surviving potential of the bovine oocytes, also very useful from a practical point of view.

Özdas et al. (2006) reported, that transportation of cattle ovaries at 4 °C is not detrimental to the in vitro development capacities of oocytes. However, the development capacity of these oocytes to blastocytes following in vitro fertilization is unknown. The development capacity fol-



HF – Holstein Frisian MB – Montbeliard

Fig. 1. Comparison of the development between the breeds (mean ± sd)

lowing IVF of these oocytes which form a model for both animals and humans must be investigated in future studies. Other possible explanations for the variability in means of different number transferable embryos could be assigned to the small number of oocytes and embryos cultured together.

Ling and Lu (1990) found no differences among the fertilization rates of different number oocytes in 50 µl drop but found differences in the developmental rates to the blastocyst stage. There is evidence that the regulation of preimplantation embryo in vitro development could be influenced by some autocrine or paracrine factors secreted by the embryos themselves (Paria et al., 1990). In the bovine the 8-, 16 cell stage block can be overcome in vitro by the use of either co-culture of embryos with helper cells or of media conditioned by these cells. Generally, the embryos are cultured in large groups and in small volumes of mediums, making use of the presumptive autocrine/paracrine factor. Embryo production from the limited number of oocytes produced by single cows could be impaired by the dilution of the autocrine/paracrine factors during embryo culture.

Conclusion

Unfortunately, the high individual variations observed in our experiments will impair the predictability of the number of transferable embryos that will be obtained from one individual slaughtered cow. In fact, in our experiments, all of the cows supplied suitable cumulus-oocyte complexes and in all cases, transferable embryos were produced. However, as mentioned above we investigated just a small group of animals. Inevitably in a largescale program, there will be cases where no suitable oocytes or transferable embryos will be produced.

The variations in our experiments could be assigned to some factors: the oocytes were matured and fertilized without selection, in small groups, there could be differences among the bulls to promote blastocyst formation. In our experiments, there were no differences between the groups making use of the same bull in promoting blastocyst development. We have no ready explanation for this phenomenon. In our experiments, we observed, that often bulls with a high AI index, in vitro are showing best results (not published). Perhaps the sperms from these bulls are with low energetical potential and the placement in the immediate vicinity by the eggs in vitro enhances the propulsion and the fertilization rates.

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