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STUDY ON QUANTITY, QUALITY AND STAGES OF DEVELOPMENT OF EMBRYOS RECOVERED FROM SUPEROVULATED COWS AND HEIFERS OF DIFFERENT BREEDS IN NORTH-EAST KAZAKHSTAN

This paper presents the research on the zygotes production, evaluation and transfer in Simmental and Kazakh whitehead cows and heifers, bred in the North-East of Kazakhstan. It was identified that, by applying the German drug «Pluset» in an optimal dose, the average numbers of embryos that can be recovered from the Simmental breed are 8,2 from a cow and 6,4 from a heifer. 66,0 % of embryos from cows were transferable, 17,0 % were non-transferable, 17,0 % of ovicells were unfertilized. Embryos recovered from heifers were identified as transferable 53,1 %, non-transferable 21,9 % and 25,0 % as unfertilized ovicells. The study showed that the embryos recovered from cows and heifers can be found in various stages of development: early morula, compact morula, early blastocyst, blastocyst, hatching and expanded blastocysts. A Kazakh whitehead cow produces 1,3 embryos more than a Simmental cow. The percentage of developed embryos was 45 % in total transplanted embryos. It was determined that the embryos transferred into heifers develop better than embryos transferred into mature cows.

Keywords: superovulation, ovary, corpus luteum, unfertilized ovicells, blastocysts, embryo, follicle, Dulbecco`s Phosphate Buffered Saline (DPBS), stage, uterus.

Introduction

Embryo quality assessment is an important factor determining the success of embryo transplantation. Embryos recovered after hormonal treatment of animals

and subsequent superovulation, have significant differences in the development of structures and by their physical, chemical and biological properties. Such diversity is observed in embryos collected not only from different donors, but also from one donor. The characteristic or quality variations in embryos is conditioned by the extended time of ovulation, a different pace of embryo development, as well as by the influence of many other internal and external factors.

The qualities of embryos resulted by superovulation depend on donors' nutrition and management factors, level of lactation, endocrine profile before ovulation, the ovarian response and the environment of uterus, individual characteristics of a donor.

There are several methods for evaluating embryos quality and viability. They are based on measuring the activity of enzymes and intensity of metabolism (glucose uptake), vital staining, measuring the bioelectric membrane potentials, in vitro cultivation, cytological and cytogenetic analysis, and others.

The most widely used method of embryo quality and viability evaluation is studying their morphological characteristics.

The development of an embryo in the uterus directly depends on the embryo quality. Embryos classified as the highest grade by their morphological traits, when transferred to recipients, develop 70 %, and those classified as satisfactory grade develop no more than 44 % [1, 2, 3]. Therefore, the goal of our research work was to determine quantity and quality of embryos recovered from several cattle breeds (Simmental and Kazakh whitehead) in North-East of Kazakhstan, and to conduct a comparative analysis.

The following objectives were defined to achieve this goal:

- to determine quantity and quality of embryos recovered from superovulated cows and heifers of Simmental and Kazakh whitehead breeds;
- through a comparative analysis, to study the stages of development of embryos from Simmental cows and heifers based on morphological indicators.

Materials and methods of research

Our research works have been conducted on Simmental and Kazakh whitehead cows in the farms of Pavlodar region over the years of 2011–2014. The selection of suitable donor cows was based on the following criteria: normal previous calving, no reproductive tract deceases, regular estrous cycle and average or above average body weight/size.

Embryos were flushed out by a catheter – model Neustadt Aisch. The uterine horn was flushed with Dulbecco Phosphate Buffered Saline (DPBS). Fluid outflow was collected in a silicone bottle. Poured into Petri dishes, the fluid with embryos was viewed under the stereo microscope Nikon SMZ at 10–20 times magnification to determine the number of embryos. After that, 50–60 times magnification was

used to evaluate embryo quality and stages of development by morphological indicators.

In accordance with stages of development the embryos were grouped into: early and compact morula and early, expanded, hatching and hatched blastocysts. Evaluated for their morphological and other traits, the embryos were characterized as: transferable, non-transferable and unfertilized ovicells.

Results of the study

Results of the study on the quantity and quality of embryos recovered from superovulated Simmental and Kazakh whitehead cattle

In order to study the influence of breed on superovulation, we selected 15 cows for our experiment, that is 8 of Simmental and 7 of the Kazakh whitehead breeds.

64 embryos and ova have been collected from the Simmental cows. That was 8 embryos and ova per cow on average; but in fact, the numbers of flushed out embryos and ova from each cow were different. For example, the largest amount of embryos and ova was 10, produced by the cow named Mary, and the smallest amount of embryos and ova was 6, produced by Izaura.

With regard to quality, 57,8 % of collected zygotes were viable and 26,6 % were non-viable. The numbers of viable and non-viable embryos per each cow also varied. In general, the proportion of viable embryos is within 55,6–66,7 % range, while the proportion of non-viable embryos is within 12,5–42,9 % range.

On average, in 15,6 % of ovicells of Simmental cows have not been fertilized. Not all the animals gave unfertilized ovicells. 6 out of 8 cows had at least 1 or 2 unfertilized ovicells, and 2 cows produced all viable embryos.

According to the results of this work we've noted that cows producing more than 8 embryos per flush, give a few unfertilized eggs (1–2), while cows producing less than 7 embryo per flush, don't have unfertilized eggs.

The results of the superovulation of Kazakh whitehead beef cows are the following: 65 embryos and eggs in total were collected from 7 experimental cows, that is, 9,3 embryos per cow, on average. 58,5 % of all embryos were transferable, 26,2 % were degenerated or undergone morphological changes, thus considered as non-transferable. 10 eggs out of 65 were unfertilized.

Results of the superovulation indicated, that Kazakh whitehead cows also have individual and physiological differences at a fairly high level. Quantities of collected embryos and eggs, ratio of transferrable and non-transferrable eggs collected from different uterus varied.

The number of flushed out embryos per cow was 8–10, transferable were 5–7 and non-transferable were from 1 to 4, 6 cows out of 7 had 1–3 unfertilized eggs and only one had none.

Table 1 below show the comparative analysis results for the superovulation of Simmental and Kazakh whitehead cows.

As shown in the chart and in the table, on average each Simmental cow produced 8,0±0,5 embryos and ova, among them 4,6±0,2 are transferable, 2,1±0,2 are non-transferable and 1,3 ± 0,3 were unfertilized. The Kazakh whitehead averaged 9,3±0,3; 5,4±0,3; 2,4±0,4 and 1,4±0,4 embryos and ova, respectively.

In general, the Kazakh whitehead breed outnumbered the Simmental breed in total production by 1,3 embryos per cow; the numbers of transferrable embryos between two breeds were basically equal. Difference between the two breeds in total flushed out embryos and in the numbers of transferable embryos is significant (p>0,95), and in unfertilized eggs number and non-transferable is insignificant.

Table 1 – Number and quality of embryos recovered from superovulated Simmental and the Kazakh whitehead cattle

Cattle breeds	Number of cows, n	Total collected embryos	Number and quality of embryos per cow		Transferable		Non-transferable		Unfertilized ova	
		n	x ± m	δ	x ± m	δ	x ± m	δ	x ± m	δ
Simmental	8	64	8,0±0,5	1,3	4,6± 0,2	0,5	2,1±0,2	0,6	1,3±0,3	0,9
Kazakh whitehead	7	65	9,3±0,3	0,9	5,4±0,3	0,9	2,4±0,4	1,0	1,4±0,4	1,0

The above data illustrate the importance of breed in inducing superovulation. Simmental is a breed of combined production and Kazakh whitehead is purely a beef breed. The difference between the two breeds is in additional productivity of Simmental.

Therefore, we can conclude that beef cattle breeds show higher results of superovulation than dairy breeds.

Results of analysis of the Simmental cattle embryos for their stage of development

Table 2 and in Diagram 2 display the results of the studies, conducted on 6-8 and 10 year old adult cattle and 18–24 month old heifers.

The analysis shows that of all the embryos collected from mature cows, 10,8 % reached the early morula stage, 40,5 % were at the compact morula stage, 29,7 % were early blastocysts, 14,9 % reached the blastocyst stage and 4,1 %

happened to be the expanded blastocysts. Consequently, 51,3 % were at the morula stage and 44,6 % reached the blastocyst stage.

This shows that morula and blastocyst develop at equal pace. Stages of development of embryo vary depending on the number of recovered embryos. A large number of early morulae and expanded blastocysts was found in the cows, which produced 9–10 more embryos, unlike in the cows which produced 7–8 embryo (early morulae and expanded blastocysts have not been found).

This conclusion is confirmed by the fact that expanded blastocyst was found in 3 out of 9 experimental cows and they gave 9–10 embryos. There is also a great variation in stages of development of embryos recovered from different cows.

For example, early morula – 10,0–25,0 %, compact morula – 33,3–50,0 %, early blastocyst – 20,0–40,2 %, the blastocyst is in the range of 11,1–25,0 %. It shows the difference between natural conditions: stimulation result in a large number of follicles, they mature at different times and the ovulation process is relatively longer, which is apparently connected, with the function of a uterine tube.

Of all the embryos collected from heifers, 10,0 % reached the early morula stage, 46,6 % – compact morula, 36,7 % – early blastocyst and 6,7 % reached the blastocyst stage. The expanded blastocysts were not there.

Thus, it should be noted that in the result of superovulation heifers do not produce expanded blastocysts, compared to adult cattle. There is also a great variation in stages of development of embryos recovered from different heifers. For example, the following figures show the lowest and highest percentages in: early morula 14,2–16,7 %, the compact morula 33,3–60,0 %, early blastocysts 28,5–42,9 %, and blastocyst 14,3–16,7 %.

Heifers which gave 6–7 embryos develop early morula compared with those which gave 5 embryos. They mostly develop the expanded blastocyst. This indicates that a large number of follicles weaken embryo development, and a small amount of them accelerates this process. This is apparently due to non-simultaneous ovulation of follicles, which causes the egg cells to release not at the same time. It in turn, affects the development of the embryo or causes delay.

Table 2 indicate the comparative analysis results of the development stages of embryos recovered from mature cows and heifers.

On average, in adult cattle early morulas amount at 10,8 %, compact morulas – at 40,5 %, early blastocysts – at 29,7 %, blastocysts – at 14,9 %, expanded blastocysts amount at 4,1 %, and in heifers, 10,0 %, 46,6 %, 36,7 % and 6,7 % respectively. This shows that the cells in mature cows and heifers mostly reach the compact morula and early blastocyst stages (mature cows 40,5 % and 29,7 %, in heifers 46,6 % and 36,7 %). The next greatest are blastocysts amounting

14,9 % in mature cows and 6,7 % in heifers. The lowest cell development is early morulas and expanded blastocysts at 10,8 % and 4,1 % respectively.

Table 2 – Comparison of embryo number by development stages in mature cows and heifers

Groups	Age	Number of animals, n	Total number of embryos		Early morula		Compact morula		Early blastocyst		Blastocyst		Expanded blastocyst	
			n	%	n	%	n	%	n	%	n	%	n	%
Mature cow	6-10 yrs	9	74	100	8	10,8	30	40,5	22	29,7	11	14,9	3	4,1
Heifer	18-24 mo	5	30	100	3	10,0	14	46,6	11	36,7	2	6,7	-	-

In heifers as compared to cows, the share of developed compact morulae and early blastocysts greater by about 13,0 % (83,3 % in heifers and 70,7 % in cows) and number of blastocyst 8,0 % more in cows. In addition, expanded blastocysts are developed more, they amount at 4,1 %. Therefore, we can conclude that the recipients, which are identified to have early estrus period, are more receptive to morulas and early blastocysts, while cows, which have late estrus, are more receptive to blastocysts and expanded blastocysts. Increasing age of cattle deteriorates the possibility to have 7 embryos; therefore the proportion of transplants is reduced, although expanded blastocyst is not found in heifers. Early morula among all embryos amounts at not less than 10,0 %, i.e. in heifers as compared to mature cows with delayed development (24 hours) early blastocysts number is less by at least once. This shows that young body has not adapted to superovulation.

Discussion

Fertilized ovum is developed in the following stages. 20–24 hours after fertilization a cell division process begins. It results a morula consisting of 16, 32 blastomeres and referred to as early morula. The next stage is compact morula consisting of 64 blastomeres. Blastocyst, depending on the level of development can be: early, expanded, hatching and hatched. After compact morula stage an embryo is developed into early blastocyst stage. Expanded blastocyst

is characterized by larger volume, narrow perivitelline space and thinned Zona pellucida. The cell masses fill the perivitelline space. Later Zona pellucida breaches and part of the cell are released. Embryo in this stage is referred to as hatching. When all the cells come out from the zona pellucida, the blastocyst is called hatched. In the normal 7-day embryo development neither hatching, nor hatched blastocysts can be found [2, 4, 5, 6].

Total embryos we collected from the experimental animals were at the following stages: 10,8 % early morula, 40,5 % compact morula, 29,7 % early blastocyst, 14,9 % blastocyst and 4,1 % the expanded blastocyst. Total morulae were 51,3 % and blastocysts were 44,6 %.

Regardless of the amount of embryos recovered from the experimental cows, embryos occur at various stages of development. However, embryos recovered in relatively larger amounts (9–10) from mature cows mostly reached the early morula and expanded blastocyst stages. In cows with smaller number of embryos early morulae and expanded blastocysts were not found.

In each mature cow, embryos are found in different stages of development: early morulae – 10,0–25,0 %, compact morulae – 33,3–50,0 %, early blastocysts – 22,3–44,4 % and blastocysts – 11,1–25,0 %.

Embryos, taken from heifers were: early morulae – 10,0 %, compact morulae – 46,2 %, early blastocyst – 36,7 %, blastocyst – 6,7 %. Expanded blastocysts were not found. Percentage of developed embryos at each stage in the experimental heifers are the following: early morulae – 14,2–16,7 %, compact morulae – 33,3–60,0 %, early blastocysts – 28,7–42,9 % and blastocysts – 14,3–16,7 %.

Heifers produced at the stage of early morulae more embryos compared with the number of embryos at the expanded blastocysts stage. Larger number of follicles in heifers slows down the development of embryo and fewer follicles create an opposite effect. We assume that acceleration or deceleration of the development of embryo is due to the fact that follicles burst out not simultaneously and therefore egg cells are released not at the same time.

On the average, mature cows produce 10,8 % – early morulae, 40,5 % – compact morulae, 29,7 % – early blastocysts, 14,9 % – blastocysts and 4,1 % – expanded blastocysts, and heifers produce 10,0 % – early morulae, 46,6 % – compact morulae, 36,7 % – early blastocysts, 6,7 % – blastocysts. It can be concluded that both mature cows and heifers produce many compact morulae and early blastocysts, 40,5 % and 29,7 %, 46,6 % and 36,7 % respectively. Early morulae and expanded blastocysts are fewer, that is 10,0 % and 4,1 % respectively.

Heifers produced roughly 13 % more of compact morulae and early blastocysts than adult cattle (83,3 % – heifers and 70,7 % – mature cows). And mature cows produced 8,0 % more blastocysts. Besides expanded blastocysts

recovered from mature cows accounted for 4,1 %. Therefore, it is recommended to transfer morulae and early blastocysts into recipient cows, which went into estrus earlier, and blastocysts and expanded blastocysts into recipient cows in later estrus.

To determine stage of development of an embryo with regard to the age of the cattle we divided our cows into 3 age groups: 6, 8 and 10 years. Despite a relatively small number of cows by age, we noticed that it does not affect the development of an embryo. 10 year old cows produce many early morulae and few blastocysts (p>0,95) comparing with the cows of other ages and heifers. 6 and 8 years old cows produce embryos at all stages of development.

The conclusion that was made is that with age, cow's reproduction ability declines and the number of produced transferable embryos reduces. In heifers also 10,0 % of early morulae are found. So, after 24 hours heifers will be able to produce up to 10,0 % of early morulae, due to neuroendocrine change in body.

Next, we would like to turn your attention to the analysis of morphological traits of the embryos collected from the donor cows.

Ernst, Sergeyev [7], Sergeyev, Amarbaev [1] collected 1512 embryos from the donor cows and heifers on the 6th and 7th day to analyze the morphology of embryos. According to the researchers among the embryos recovered on the 6th day, 27,4 % were early morulae, 69,3 % were morulae and 3,3 % reached the early blastocyst stage. On the 7th day, number of morulae decreased and number of blastocysts increased. It should be noted that the numbers of embryos by stages of development changed: early morulae by 9, morulae by 10,2, early blastocysts by 79,5, expanded blastocysts by 1,3 %. 38,2 % of tested embryo, from the morphological point of view, are formed and comply with all stages of development. Alongside with that, 24,1% were degenerated embryos and 37,7 % comprised unfertilized ova. Cows had more fertilized embryos than heifers, that is, 33,6 % and 46,8 %. Meanwhile heifers had more unfertilized ova than cows, 42,8 % and 28,4 % respectively.

Among zygotes collected from 1116 beef and dairy cows, 58,0 % were transferable embryos, 31,0 % unfertilized ova and 11,0 % non-transferable embryos [8].

M. Ayatkhanuly, K. Landing, H-P Nohner [9, 10] collected 791 embryos from 47 Simmental donor cows and analyzed them by their stages of development. Of total embryos, 63,8 % were transferable, 15,1 % were non-transferable and 21,1 % were unfertilized ova. Zygotes recovered 7 days after the superovulation, have been grouped as follows: earlier morulae – 22,8 %, morulae – 49,62 %, early blastocyst – 16,26 %, expanded blastocyst – 11,32 %.

Jutta Schwab [11] used follitropin to stimulate superovulation in 202 donors. The result was 69,9 % of transferable zygotes. Out of these, 76,6 % were morulae and 23,4 % were blastocysts.

According to the referenced study works, treatment with gonadotropin can always cause superovulation and the development of embryos at all stages. All studies confirm that majority of flushed out embryos is the compact morulae and early blastocysts. However, it does not mean that there are no early morulae, expanded, hatching or hatched blastocysts. Compared to natural conditions it may be said that deceleration or acceleration of superovulation is associated with a large number of follicles in the ovaries.

A large number of follicles cannot grow simultaneously: some develop fast, the others grow normally, and some develop with delay. Differently growing follicles result in non-simultaneous release of eggs, and cause the ovulation process to take 4-12 hours longer [12]. Sperm and ovum unite, influencing the process of fertilization. Under their influence, the zygote can change its movement in one or another direction. Hormonal imbalance causes changes in uterus. The mentioned factors constituting superovulation of cows and heifers drive changes in the formation of embryo. Age, type used hormone, cattle breed and a number of other factors also play an important role in the process superovulation.

Conclusions

Our studies on the Simmental and Kazakh whitehead cattle breeds in Pavlodar region allow the following conclusions:

1. Total 65 embryos and egg cells have been recovered from the Simmental cows. The average number of embryos found per cow was 8 Out of total zygotes, 57,8 % were transferable and 26,6 % were non-transferable. On average, 15,6 % of eggs were unfertilized.

2. 65 embryos and egg cells have been recovered from the Kazakh whitehead cattle, that is 9,3 embryos per cow, on average. 58,5 % of flushed embryos were transferable and 26,2 % of embryos had not undergo morphological or other changes and that is why referred to as non-transferable. 10 eggs out of 65 were unfertilized.

3. Total embryos collected from the Simmental cows developed the following stages in percentage: 10,8 % – early morula, 40,5 % – compact morula, 29,7 % – early blastocyst, 14,9 % – blastocyst, 4,1 % – expanded blastocyst. In general, morulae comprised 51,3 % and blastocysts comprises 44,6 %.

3. Total embryos collected from heifers of the Simmental breed are grouped as: 10,0 % – early morula, 46,6 % – compact morula, 36,7 % – early blastocyst, 6,7 % – blastocysts. Expanded blastocysts were not found. Thus, it should be noted

that superovulated heifers do not produce expanded blastocysts as compared to mature cows.

4. There is also a great variation in stages of development of embryos recovered from different heifers. For example, the following figures show the lowest and highest percentages in: early morula 14,2–16,7 %, the compact morula 33,3–60,0 %, early blastocysts 28,5–42,9 %, and blastocyst 14,3–16,7 %.

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ИЗУЧЕНИЕ КОЛИЧЕСТВА, КАЧЕСТВА И СТАДИИ РАЗВИТИЯ ЭМБРИОНОВ ОТ СУПЕРОВУЛИРОВАННЫХ КОРОВ И ТЕЛОК РАЗНЫХ ПОРОД В УСЛОВИЯХ СЕВЕРО-ВОСТОЧНОГО КАЗАХСТАНА

В этой статье впервые была проведена работа по получению зигот, оценка и пересадка на коровах и телках симментальской и казахской белоголовой пород, разводимых на северо-востоке Казахстана. Было выяснено, что путем применения Германского препарата «Плусет» в оптимальной дозе можно получить в среднем от коров-доноров симментальской породы 8,2, от телок 6,4 эмбрионов. 66,0 % полученных эмбрионов коров были пригодными к пересадке, а 17,0 % непригодными, кроме того, 17,0 % яйцеклеток оказались неоплодотворенными. Из всех эмбрионов телок 53,1 % были пригодными, 21,9 % были непригодными, 25,0 % были неоплодотворенными яйцеклетками. У коров и телок можно встретить эмбрионы на различных стадиях развития: ранняя морула, компактная морула, ранняя бластоциста, бластоциста, выступившие и расширенные бластоцисты. По сравнению с симментальской породой у коров белоголовой породы можно получить на 1,3 эмбриона больше. Доля развитых из всех пересаженных эмбрионов составило 45,5 %. Было определено, что пересаженные телкам эмбрионы развиваются лучше по сравнению с коровами.

Ключевые слова: суперовуляция, овуляция, желтое тело, бластоциста, эмбрион, фолликула, Физиологический раствор с фосфатным буфером Дульбекко (DPBS), стадия, матки.

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СОЛТҮСТІК-ШЫҒЫС ҚАЗАҚСТАН ЖАҒДАЙЫНДА СУПЕРОВУЛЯЦИЯ ТҮЗІЛТКЕН УӘРТҮРЛІ ТҰҚЫМДЫ СИЫРЛАР МЕН ҚҰНАЖЫНДАРДЫҢ ЭМБРИОНДАРЫНЫҢ САНЫН, САПАСЫН ЖӘНЕ ДАМУ САТЫСЫН ЗЕРТТЕУ

Бұл мақалада алғаш рет Қазақстанның солтүстік-шығысында өсірілетін симментал және қазақтың ақбас тұқымдарының сиырлары мен құнажындарынан эмбрион шайып алу, бағалау және көшірі отырғызу жұмыстары жүргізілді. Германдық «Плусет» препаратын оңтайлы дозада қолдану арқылы орта есеппен симментал тұқымының донор сиырларынан 8,2, құнажындарынан 6,4 эмбрион алуға болатындығы анықталды. Алынған сиыр эмбриондарының 66,0 %- ы трансплантацияға жарамды, ал 17,0 %-ы жарамсыз, сонымен қатар аналық клеткалардың 17,0 %-ы ұрықтанбаған. Барлық құнажындардың эмбриондарының 53,1 %- ы жарамды, 21,9 %-ы жарамсыз, 25,0 %-ы ұрықтанбаған аналық торшасы болды. Сиырлар мен құнажындар эмбриондарының даму сатылары әртүрлі кезеңдерінде кездестіруге болады: ерте морула, ныгыз морула, ерте бластоциста, бластоциста, жылтиған және керілген бластоцисталар. Симментал тұқымымен салыстырғанда, ақ бас сиырларынан 1,3 көп эмбрион алуға болады. Барлық көшіріліп отырғызылған эмбриондардың дамыған үлесі 45,5 % құрады. Құнажындарға трансплантацияланған эмбриондар сиырларға қарағанда жақсы дамитыны анықталды.

Кілтті сөздер: суперовуляция, овуляция, сары дене, бластоциста, эмбрион, фолликула, фосфат буфері бар физиологиялық Дюльбекко ерітіндісі, сатысы, жатыр.

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