Comparative evaluation of the efficiency of poliovulation induction in donor cows using "FSH-SUPER" drug with various injection schemes

Artem Brigida
Department of Experimental Transplantology
Center of Experimental Embryology and Reproductive Biotechnologies
Moskow, Russia
brigida_86@mail.ru

Olga Skachkova
Scientific and Organizational Department
Center of Experimental Embryology and Reproductive Biotechnologies
Moskow, Russia
oaskachkova@mail.ru

Olga Bykova
Department of technologies of production and processing of agricultural products
Ural State Agrarian University
Ekaterinburg, Russia
olbyk75@mail.ru

Vladimir Sorokin
Department of Non-communicable Diseases
Orenburg State Agrarian University
Orenburg, Russia
vis5256@yandex.ru

Abstract— **This article describes the problems of traditional poliovulation induction scheme with repeated injections of follicle-stimulating hormone (FSH) to embryo donor cows. The purpose of this study was a comparative analysis of poliovulation induction results in donors received one or several FSH injections. Experimental animals (n = 256) in group I (n = 96) received injections of "FSH-super" drug eight times, every 12 hours for 4 days; in group II (n = 73) there was one injection of "FSH-super" combined with polyvinyl alcohol with a molecular weight of 15,000 Da; in group III (n = 87) there was one injection of "FSH-super" combined with polyethylene glycol with a molecular weight of 6,000 Da. The number of donor cows with positive response to hormonal treatment in groups I-III was similar: 94.8%, 95.9%, and 95.4%, respectively, what shows the effectiveness of one injection of long acting FSH. At the same time, group III (with polyethylene glycol as prolongator) was the leader in the effectiveness of induced poliovulation – here the highest ovarian poliovulatory reaction was obtained with the formation of 15.2 yellow bodies per one donor (in groups II and I – 14.0 and 10.5, respectively). The number of obtained proper embryos in group III was higher by 9.6% than in group II, and by 15.6% than in group I, while the number of degenerated embryos in group III was 2.5% less than in group II and 15.0% less than in group I. In group III, there were the highest level of ovum fertilization rate (82.2%), the largest number of excellent- and good-quality embryos (optimal for cryopreservation) – 89.9%, and also, of embryos at the stages of late morula, early blastocyst and expanded blastocyst (optimal for transplantation) – 93.3%. The considered facts can be taken into account when developing protocols for one FSH injection in order to stimulate poliovulation in embryo donor cows.**

Keywords— *donor cow, poliovulation induction, follicle-stimulating hormone, prolongator, polyethylene glycol, polyvinyl alcohol, maximum ovulations, embryo.*

I. Introduction

Embryo transplantation technology is one of the main biotechnological methods for quick cattle reproduction. More than one million embryos are produced in the world annually, while approximately half of them are obtained *in vivo* from donor cows. World experience shows that, at present, the average yield of proper embryos for induction of ovarian poliovulation in donor cows is 6-8 (per one donor).

For example, according to AETE (Association of embryo technology in Europe, 2019) data, in European countries involved in cattle embryo transplantation, in 2017, the average yield of such embryos per donor was 6.4, in particular, in the Netherlands 8.8, in the Russian Federation – 6.9, in Serbia – 4.2 [1]. However, with ever-increasing interest in intensifying herd reproduction using reproductive biotechnologies, these figures are rather small compared with the fact that there are hundreds of thousands of antral follicles with immature germ cells (ova) in cows' ovaries, the physiological maximum of which cannot be exhausted using the existing methods of poliovulation induction with exogenous gonadotropins. Currently developed protocols for poliovulation stimulation in donor cows do not make it possible to fully activate the multiple growth and development of follicles. So, using of classical protocol which is widely applied in modern veterinary practice and is based on the repeated injections of follicle-stimulating hormone (8-10 injections, every 12 hours for 4-5 days) makes it possible to obtain such negative results as the absence of poliovulation (approximately 20-30% of treated animals do not respond to hormonal stimulation) or poor ovulation result (from 1 to 3 embryos obtained from the other 20-30% of treated animals) [2]. Special attention of researchers was attracted by the possibility of rejection of repeated injections of follicle-stimulating hormone (FSH) due to the unsatisfactory poliovulation, as well as significant labor effort and working time during this protocol. Researches are being conducted around the world, which are aimed at developing new schemes and regimens for FSH-drugs administration in order to obtain more effective results of poliovulation induction in donor cows. One of the directions is studying the possibility of one injection of this hormone which may cause an increase in the number of simultaneously growing and maturing follicles with the release of proper ova. The hypothesis of an increase in the level of the poliovulatory reaction in the ovaries of donor cows in a similar way was partially confirmed by separate studies conducted with substances prolonging the action of FSH for 4-5 days (time sufficient for the simultaneous maturation of fully functioning ova) with one injection. In particular, an increase in the number of embryos suitable for transplantation by 1.2 was reported for the experimental group of donor cows where FSH was once injected, together

with the polyvinyl alcohol (PVA) as a prolongator, in comparison with the control group of animals where 8 injections of this gonadotropin were made (7.5 vs. 6.3) [3]. Other reference sources contain the information that by combining polyethylene glycol (PEG) polymer with follicle-stimulating hormone injected into the body of mammal, the rate of FSH release can be controlled during several days [4]. Information in the world literature about the effectiveness of using substances prolonging FSH action is rare and sketchy with regard to time periods [5-7], but, nevertheless, there is a chance of positive effect on the increase in the number of maturing follicles in donor cows, and, consequently, the increase the number of viable embryos which can completely develop after their transfer to the reproductive system of recipient cows. At the moment, the issue of one FSH injection in combination with polyvinyl alcohol or polyethylene glycol as prolongators has not been sufficiently studied, there is no information about protocols that clearly regulate the regimens and dosages of administration for this hormone in combination with prolongators, and also indicate the range of expected results by the number of cattle embryos obtained.

Based on the foregoing, the purpose of this study was to study the effectiveness of poliovulation induction in embryo donor cows using "FSH-super" drug under various schemes of its administration, including using substances which can prolong the effect of exogenous FSH of pituitary origin in the animal.

II. METHODS AND MATERIALS

For this study, we selected 256 cows as potential embryo donors. Ovary cycle of embryo donor cows was induced with GNRH analogues – 5-10 ml i.m. and PgF2a cloprostenol at the dosis of 500 μg per cow i.m.

Poliovulation induction in donors was performed using a gonadotropic pituitary drug "FSH-super" (manufacturer – Russia) with follicle-stimulating hormone activity of 1000 IU (50 Armour units, AU) which is sufficient to stimulate the physiological growth and maturation of many follicles and ova for fertilization and developing into proper embryos.

All animals were divided into three groups, depending on a hormonal stimulation scheme used.

In group I (n = 96), in donor cows on the 9-12 day of ovary cycle, a protocol was used based on eight intramuscular injections of "FSH-super" drug with an interval of 12 hours (morning-evening) in decreasing doses (50 AU, 3; 3; 2.5; 2.5; 2; 2; 1.5; 1.5 ml/cow.). In groups II and III, one injection of "FSH-super" was used in combination with a substance prolonging FSH action. In group II (n = 73), each donor cow on the tenth day of ovary cycle once received, subcutaneously in the area of scapula, a mixture consisting of "FSH-super" (FSH in full – 50 AU), a prolongator – polyvinyl alcohol (PVA) with molecular weight of 15,000 Da at the rate of 0.9 grams per cow, and solvent – saline solution (0.9% aqueous solution of sodium chloride) in a volume of 10 mL. In group III (n = 87), each donor cow on the tenth day of ovary cycle once received, subcutaneously in the area of scapula, a mixture consisting of "FSH-super" (FSH in full – 50 AU), a prolongator – polyethylene glycol (PEG) with molecular weight of 6,000 Da at the rate of 3 grams per cow, and solvent –saline solution (0.9% aqueous solution of sodium chloride) in a volume of 10 mL.

Insemination of donor cows was carried out at the time estrus start, three times, with an interval of 12 hours using five doses of semen; rectocervical insemination method was used.

On the seventh day from the first insemination, embryos were retrieved from the reproductive organs of donor cows. Immediately prior to the retrieval of embryos from donor cows, the number of yellow bodies on the surface of ovaries was counted using the recto-palpatory method and/or echographic imaging with EASI SCAN E4 ultrasound scanner ("BCF Technology", United Kingdom). At the same time, the number of counted yellow bodies (the number of ovulations) was considered as equivalent to the number of matured and ovulated follicles from which the ova came out.

Retrieval of embryos was carried out using a 60 ml Luer syringe. For washing out the embryos, flexible two- and three-lumen catheters were used as auxiliary equipment for retrieval of embryos from the Foley tips manufactured by "Minitube" company (Germany). Dulbecco's balanced buffered saline was used as a washing liquid, with the addition of 10% blood serum, in the total volume of 500 ml per uterine horn. Filters for collecting embryos manufactured by "Minitube" company (Germany) were used for filtering the washing fluid with retrieved embryos.

A microscopic study of obtained embryo retrievals was carried out, in this process a MBS-10 stereo microscope with MFU micrographic device and VEC-545-USB television camera (AO "Lytkarinsky Optical Glass Plant", Russia) with magnification of 60-100 times and more was used for search and morphological assessment of retrieved embryos. The number of proper embryos (suitable for transplantation or cryopreservation) at different developmental stages (early and late morula, early blastocyst, expanded or fully expanded blastocyst), and the number of degenerated embryos and unfertilized ova were counted. Morphological assessment of embryos took into account the compliance with developmental stages, the shape of pellucid zone and its integrity, the equability of blastomere cleavage ,and the general state of cytoplasm according to GOST 28424-2014 (2015). Particular attention was paid to the transparency of perivitelline space and polygonal connection between blastomeres.

The results of these studies are presented in four tables taking into account the gradation of animals within experimental groups according to maximum ovulations: animals with 3-5, 6-10, 11-20 yellow bodies and with more than 20 yellow bodies in ovaries.

III. RESULTS

Evaluation of the results obtained during poliovulation induction in donor cows of experimental groups shows that of the 256 animals that underwent poliovulation stimulation, only 244 donor cows had positive response, i.e. three or more yellow bodies in ovaries. The remaining 11 animals either had no poliovulation (the number of ovulations – 0 yellow bodies in ovaries), or showed low poliovulation (the number of ovulations – 1-2 yellow bodies in ovaries) which in this experiment was equated to the absence of poliovulation due to the fact that cows and heifers normally produce 1-2 yellow bodies with the natural course of ovary cycle. As we can see from Table 1, in group I (n = 96) there was a positive poliovulatory response in 91 animals (94.8%), in group II (n = 73) – in 70 animals (95.9%), in group III (n = 87) – in 83

animals (95.4%). It follows that in all three groups there was a slight difference in the number of treated animals that did not respond to hormonal stimulation (in group I there was no response to injected gonadotropins in 5.2% of animals, in group II – in 4.1%, in group III – in 4.6% of animals). These data demonstrate that one FSH injection in combination with a prolongator (PVA in group II, PEG in group III) does not reduce the number of animals with positive response to hormonal treatment in comparison with the standard protocol of repeated FSH injections during 4 days which was used in group I.

TABLE I. QUANTITATIVE PARAMETERS OF THE RESULTS OF POLIOVULATION INDUCTION AND PARAMETERS OF EMBRYO RETRIEVAL (X±Sx)

Group, method of FSH administration	Donors with positive response to poliovulation induction, n / %	Maximum ovulations, lim	Donors distributed according to maximum ovulations, n / %	Yellow bodies, n		Proper degenerated embryos, unfertilized ova (total number), n		Proper embryos	
				Total	Per donor	Total	Per donor	Total, n / %	Per donor, n
I, n = 96 (eight injections)	91 / 94.8	3 - 5	10/11.0	36	3.6±0.26	30	3.0±0.76	13/43.3	1.3±0.37
		6-10	42/46.1	315	7.5±0.24	261	6.2±0.33	135/51.7	3.2±0.29
		11-20	31/34.1	400	12.9±0.54	319	10.3±0.54	177/55.5	5.7±0.44
		>20	8/8.8	205	25.6±1.43	172	21.5±1.11	97/56.4	12.1±2.12
Average for group I			91	956	10.5±0.69	774	8.5±0.59	422/54.5	4.6±0.41
II, n = 73 (one FSH injection + PVA)	70 / 95.9	3 - 5	6/8.5	25	4.2±0.37	22	3.7±0.24	11/50	1.8±0.20
		6-10	23/32.9	202	8.8±0.31	159	6.9±0.46	87/54.7	3.8±0.37
		11-20	32/45.7	518	16.2±0.56	442	13.8±0.48	250/56.6	7.8±0.54
		>20	9/12.9	234	26.0±0.74	211	23.4±0.72	152//72.0	16.9±1.24
Average for group II			70	979	14.0±0.89	826	11.8±0.83	500/60.5	7.1±0.66
III, n=87 (one FSH injection + PEG)	83 / 95.4	3 - 5	8/9.6	33	4.1±0.31	32	4.0±0.26	18 / 56,3	2,3±0,48
		6-10	22/26.5	198	9.0±0.24	156	7.1±0.43	121 / 77,6	5,5±0,41
		11-20	40/48.2	676	16.9±0.42	568	14.2±0.53	388 / 68,3	9,7±0,58
		>20	13/15.7	355	27.3±0.62	309	23.8±1.00	224 / 72,5	17,2±1,02
Average for group III			83	1262	15.2±0.92	1065	12.8±0.85	751 / 70.5	9.0±0.66

When considering "the number of donors distributed according to maximum ovulations" parameter, the following trend was revealed: in group II (FSH injection combined with PVA) and in group III (FSH injection combined with PEG) in comparison with group I (8 FSH injections), an increase in the number of animals with maximum ovulations of 11-20 and with >20 yellow bodies in ovaries was noted (Table 1). The number of donors with indicated parameters counted as a whole was 42.9% in group I, 58.6% in group II, and 63.9% in group III. Thus, in group II compared with group I, the number of donors with indicated parameters (as a whole) was 15.7% more, and in group III compared to group I, the same value was 21.0% more. At the same time, the same indicator in group III as compared with group II was 5.3% more. In this regard, it is possible to say that with one FSH injection together with PVA or PEG as a prolongator, the number of animals with higher level of poliovulatory reaction increased (11-20 and >20 yellow bodies in ovaries).

Turning to the consideration of "yellow bodies" formed in ovaries after poliovulation induction (Table 1), we note that in group I the average number of yellow bodies in ovaries (number of ovulations) per donor was 10.5, in group II – 14.0, and in group III – 15.2 what shows the formation of a greater number of yellow bodies in animals of group III

compared with similar parameters of group II and I – by 1.2 and 4.7, respectively. At the same time, there was a statistically significant increase (p <0.05) in the number of ovulations between groups III and I.

Estimating the parameters of obtained embryo retrievals when the total number of proper embryos, degenerated embryos and unfertilized ova found by microscopic examination was counted, the values obtained by recalculating their total number per donor were taken into account. In group I, such value per one donor was 8.5 on average for this group, 11.8 for group II, and 12.8 for group III (Table 1). This demonstrates the fact that the parameter of group III in the number of such embryos and unfertilized ova exceeds the similar value of group II by 1.0, and of group I – by 4.3 pcs. At the same time, in group III, there was a significant increase (p <0.01) in the number of embryos and unfertilized ova (as a whole) in comparison with the similar parameters in group I. In comparison with group II, there were no significant differences.

A separate calculation of the number of proper embryos showed that when recalculating the number of proper embryos per donor in group I, the average value for this group was 4.6, for group II – 7.1, and for group III – 9.0. This parameter was based on the total number of proper embryos, degenerated embryos and unfertilized ova: in group

I – 54.5%, in group II – 60.5%, and in group III – 70.1% (Tables 1, 2). This shows that in group III, we received by 9.6% more proper embryos than in group II, and by 15.6% more than in group I; and in group II, proper embryos were received by 6.0% more than in group I.

TABLE II.   QUANTITATIVE PARAMETERS OF EMBRYO RETRIEVAL AND OVUM FERTILIZATION RATE IN POLIOVULATORY DONOR COWS (X±Sx)

Group with positive response to poliovulation induction, donors, n (administration method, FSH)	Maximum ovulations, lim	Parameters of embryo retrieval						Fertilization rate	
		Proper embryos		*Degenerated embryos*		*Unfertilized ova*			
		Total, n/%	*Per donor, n*	*Total, n/%*	*Per donor, n*	*Total, n/%*	*Per donor, n*	*Total, n*	*Per donor, %*
I, n = 91 (eight injections)	3 - 5	13/43.3	1.3±0.37	10/33.3	1.0±0.27	7/23.3	0.7±0.25	2.3	76,7
	6-10	135/51.7	3.2±0.29	75/28.7	1.8±0.23	51/19.5	1.2±0.23	5.0	80,5
	11-20	177/55.5	5.7±0.44	86/27.0	2.8±0.32	56/17.6	1.8±0.32	8.5	82,5
	>20	97/56.4	12.1±2.12	39/22.7	4.9 ±0.70	36/20.9	4.5±0.96	17.0	79,1
Average for group I		422/54.5	4.6±0.41	209/27.0	2.3±0.20	143/18.5	2.0±0.20	6.9	81.5
II, n = 70 (one FSH injection + PVA)		11/50	1.8±0.20	6/27.3	1.0±0.32	5/22.7	0.8±0.2	2.7	77.7
		87/54.7	3.8±0.37	16/10.1	0.7±0.19	56/35.1	2.4±0.33	4.5	64.8
		250/56.6	7.8±0.54	74/16.7	2.3±0.45	118/26.7	3.7±0.11	10.1	73.3
	>20	152//72.0	16.9±1.24	24/11.4	2.7±0.71	35/16.6	3.9±1.26	19.6	83.4
Average for group II		500/60,5	7.1±0.66	120/14.5	1.7±0.27	214/25.9	3.1±0.26	8.9	74.3
III, n =83 (one FSH injection + PEG)	3 - 5	18 / 56.3	2.3±0.48	9/28.1	1.1±0.31	5/15.6	0.6±0.33	3.4	84.4
	6-10	121 / 77.6	5.5±0.41	15/9.6	0.7±0.22	20/12.8	0.9±0.26	6.2	87,2
	11-20	388 / 68.3	9.7±0.58	72/12.7	1.8±0.24	108/19.0	2.7±0.35	11.5	81,0
	>20	224 / 72.5	17.2±1.02	32/10.4	2.5±0.60	53/17.2	4.1±0.87	19.7	82,9
Average for group III		751 / 70.5	9.0±0.66	128/12.0	1.5±0.18	190/17.8	2.3±0.27	10.5	82.2

A separate calculation of the number of degenerated embryos showed that when recalculating the number of degenerated embryos per donor in group I, the average for this group was 2.3, for group II – 1.7, and for group III – 1.5. When re-counting from the total number of proper embryos, degenerated embryos and unfertilized ova, the index of the number of degenerated embryos was: in group I – 27.0%, in group II – 14.5%, and in group III – 12.0% (Table 2). At the same time, in group III degenerated embryos were obtained by 2.5% less than in group II and 15.0% less than in group I. In group II, degenerated embryos were obtained by 12.5% less than in group I. In terms of degenerated embryos, a statistically significant difference ($p < 0.05$) was found between parameters of group III and group I. There were no significant differences compared with group II.

Taking into account the number of degenerated embryos in respect of maximum ovulations, we can note that in groups II and III the highest number of degenerated embryos was recorded with maximum ovulations of 3-5 yellow bodies in ovaries, and with maximum ovulations of 6 -10, 11-20 and >20 yellow bodies in ovaries the number of such embryos was significantly smaller. At the same time, the number of degenerated embryos in group I was approximately the same with all maximum ovulations (3-5, 6-10, 11-20 and >20 yellow bodies in ovaries). Thus, when using one FSH injection together with polyvinyl alcohol in group II and polyethylene glycol in group III as prolongators, a decrease in the number of degenerated embryos in animals with the response of 6 or more yellow bodies was observed. This fact, in comparison with the repeated FSH injections in group I, indicates a more uniform growth and maturation of follicles with the formation of proper ova for fertilization with the subsequent formation of fully functional embryos at the early stages of development.

Separate counting of the number of unfertilized ova showed that when re-counting the number of unfertilized ova per donor in group I, the average for this group was 2.0, for group II – 3.1, and for group III – 2.3. When re-counting from the total number of proper embryos, degenerated embryos and unfertilized ova, the number of unfertilized ova was: in group I – 18.5%, in group II – 25.9%, and in group III – 17.8% (Table 2). It should be noted that the number of unfertilized ova during embryo retrieval was taken into account, in the framework of this study, as a criterion for determining ovum fertilization rate in the course of conducting poliovulation induction. At the same time, ovum fertilization rate meant the percentage of fertilized ova from the total number of ova released from the follicles of donor cows as a result of poliovulation caused by exogenous gonadotropins. Based on the data obtained, the average fertilization rate was found for all three groups. In group I, fertilization rate percentage was 81.5%, in group II – 74.3%, and in group III – 82.2% (Table 2). As we can see from the above data, group III where polyethylene glycol was used as a prolongator showed the highest ovum fertilization rate. At the same time, the lowest fertilization rate percentage was observed in group II where polyvinyl alcohol was used as a prolongator.

Thus, as a result of the above analysis conducted in all three experimental groups on the total number of proper embryos, degenerated embryos and unfertilized ova found during the microscopic study, as well as on their separate count, the following was established. In group III (one FSH injection combined with PEG), there was not only the increase in the number of proper embryos by 15.6%, but also the increase in ovum fertilization rate by 0.7% in comparison with the values of same parameters in group I where the protocol of repeated FSH injections was used. In group II (one FSH injection combined with PVA) in comparison with group I, the increase in number of proper embryos by 6.0% was also observed, but with a decrease in ovum fertilization rate by 7.2%. When comparing these parameters between groups II and III, it was revealed that in group III there were obtained more proper embryos by 9.6% than in group II, and the increase in ovum fertilization rate by 7.9% was recorded.

In addition, in the framework of this research, the study of embryo retrieval included a gradation of proper embryos using a quality assessment scale (embryos could be excellent, good, satisfactory, conditionally suitable, unsuitable), while all degenerated embryos were found to be unsuitable and

should be rejected. Studying the results of differentiation of proper embryos during embryo retrieval in accordance with the quality assessment scale was necessary due to the fact that all proper embryos were suitable for transplanting in a freshly obtained form, and only excellent- and good-quality embryos can be used for embryo preservation with cryopreservation method (Table 3).

TABLE III. PARAMETERS OF THE NUMBER OF PROPER EMBRYOS DISTRIBUTED IN ACCORDANCE WITH QUALITY ASSESSMENT SCALE (X±Sx)

| Group with positive response to poliovulation induction, donors, n (administration method, FSH) | Maximum ovulations, lim | Proper embryos | | Proper embryos evaluated using quality assessment scale | | | | | | | |
| | | | | Excellent | | Good | | Satisfactory | | Conditionally suitable | |
		Total, n/%	Per donor, n	Total, n/%	Per donor, n	Total, n/%	Per donor, n	Total, n/%	Per donor, n	Total, n/%	Per donor, n
I, n = 91 (eight injections)	3 - 5	13/43.3	1.3±0.37	2/15.4	0.2± 0.16	8/61.5	0.8± 0.31	1/7.7	0.1± 0.13	2/15.4	0.2±0.13
	6-10	135/51.7	3.2±0.29	35/25.9	0.8± 0.19	70/51.9	1.7± 0.20	22/16.3	0.5± 0.18	8/5.9	0.2± 0.07
	11-20	177/55.5	5.7±0.44	38/21.5	1.2± 0.26	98/55.4	3.2± 0.31	26/14.7	0.8± 0.24	15/8.5	0.4± 0.17
	>20	97/56.4	12.1±2.12	23/23.7	2.9± 0.70	48/49.5	6.0± 1.16	15/15.5	1.9± 1.48	11/11.3	1.4± 0.56
Average for group I		422/54,5	4.6±0.41	98/23.2	1.3±0.32	224/53.1	2.9±0.48	64/15.2	0.8±0.24	36/8.5	0.6±0.12
II, n = 70 (one FSH injection + PVA)	3 - 5	11/50	1.8±0.20	4/36.4	0.7± 0.25	6/54.6	1.0±0.32	1/9.1	0.2± 0.21	----	----
	6-10	87/54.7	3.8±0.37	37/42.5	1.6± 0.32	42/48.3	1.8± 0.38	5/5.8	0.2± 0.10	3/3.4	0.1± 0.76
	11-20	250/56.6	7.8±0.54	97/38.8	3.0±0.49	127/50.8	4.0± 0.74	18/7.2	0.6± 0.16	8/3.2	0.2± 0.12
	>20	152//72.0	16.9±1.24	53/34.9	5.9± 172	73/48.0	8.1± 1.17	16/10.5	1.7± 0.64	10/6.6	1.1± 0.41
Average for group II		500/60,5	7.1±0.66	19.1/38.2	2.8±0.38	248/49.6	3.7±0.44	40/8.0	0.7±0.36	21/4.2	0.4±0.24
III, n =83 (one FSH injection + PEG)	3 - 5	18 / 56.3	2.3±0.48	7/38.9	0.9± 0.48	8/44.4	1.0± 0.12	3/16.7	0.4± 0.21	----	----
	6-10	121 / 77.6	5.5±0.41	48/39.7	2.2± 0.57	61/50.4	2.8± 0.32	11/9.1	0.5± 0.22	1/0.8	0.1± 0.06
	11-20	388 / 68.3	9.7±0.58	198/51.0	5.0± 0.50	140/36.1	3.5± 0.40	43/11.1	1.1± 0.26	7/1.8	0.2± 0.07
	>20	224 / 72.5	17.2±1.02	94/42.0	7.2± 0.68	119/53.1	9.2± 0.72	7/3.1	0.6± 0.24	4/1.8	0.3± 0.24
Average for group III		751 / 70,5	9.0±0.66	347/46.2	3.8±0.57	328/43.7	4.1±0.47	64/8.5	0.7±0.33	12/1.6	0.2±0.11

As we can see from Table 3, the number of embryos of excellent quality counted on average in group III (46.2%) exceeded the average number of such embryos in group II (38.2%) by 8.0%, and in group I (23 , 2%) – by 23.0%; in group II the number of such embryos was higher than in group I by 15.0%. Statistically significant difference (p<0.05) between the parameters of group III and group I was established in terms of excellent quality embryos. In comparison with group II, no significant differences were noted. In addition, when calculating embryos suitable for cryopreservation (excellent and good quality) in group III, the number of such embryos was also exceeded amounting to 89.9% of the total number of proper embryos in this group, on average, compared with group II (87, 8%) by 2.1%, and with group I (76.3%) – by 13.6%. Also, when calculating embryos estimated on a quality scale as satisfactory and conditionally suitable, it was found that in group III (on average for this group) there were significantly less such embryos compared with other groups. At the same time, in group III there were 10.1% such embryos, in group II – 12.2% and in group I – 23.7%. Thus, in group III where one FSH injection was used together with PEG as a prolongator, the highest number of proper embryos which were classified as excellent and good ones, and the minimum number of satisfactory and conditionally suitable embryos were obtained in comparison with group II where one FSH injection was used together with PVA as a prolongator, as well as with group I where eight FSH injections were used.

In the course of this study, the influence of used gonadotropic treatment schemes at the stage of embryo development in donor cows was also assessed (Table 4). For this end, the number of proper embryos in obtained embryo retrievals was counted, depending on the stage of their development: early morula; late morula; early blastocyst; expanded blastocyst; fully expanded blastocyst. This embryo gradation was used to study the synchronicity of embryo development indicating the synchronicity of the ovulatory response of many follicles caused by poliovulation induction with gonadotropic drugs. At the same time, special attention was paid to the number of embryos that were in the stages of late morula, early blastocyst and expanded blastocyst, due to the fact that these stages are the most favorable from the point of view of transplanted embryo survival in recipient body. Embryos that are at other stages of development (early morula, fully expanded blastocyst), despite the fact that they are suitable for transplantation to recipients, however, require more precise compliance and synchronization with the ovary cycle of recipient at each of these stages of embryo development.

TABLE IV.   PARAMETERS OF THE NUMBER OF PROPER EMBRYOS DISTRIBUTED IN ACCORDANCE WITH THE CLASSIFICATION OF MATURITY STAGES (X±Sx)

| Group with positive response to poliovulation induction, donors, n (administration method, FSH) | Maximum ovulations, lim | Maturity stages of proper embryos | | | | | | | | | |
| | | Early morula | | Late morula | | Early blastocyst | | Expanded blastocyst | | Fully expanded blastocyst | |
		Total, n/%	Per donor, n	Total, n/%	Per donor, n	Total, n/%	Per donor, n	Total, n/%	Per donor, n	Total, n/%	Per donor, n
I, n = 91 (eight injections)	3 - 5	1/7.7	0.10±0.125	7/53.8	0.70±0.263	3/23.1	0.30±0.183	2/15.4	0.20±0.125	----	----
	6-10	20/14.8	0.48±0.128	34/25.2	0.81±0.173	42/31.1	1.00±0.211	26/19.3	0.62±0.158	13/9.6	0,32±0,109
	11-20	27/15.3	0.87±0.233	39/22.0	1.26±0.202	52/29.4	1.68±0.256	36/20.3	1.16±0.206	23/13.0	0,77±0,241
	>20	15/15.5	1.88±0.307	21/21.7	2.63±0.333	31/32.0	3.88±0.543	19/19.6	2.38±0.615	11/11.3	1,5±0,806
Average for group I		63/14,9	0.83±0.233	101/23.9	1.35±0.244	128/30.3	1.72±0.64	83/19.7	1.09±0.233	47/11.2	0.65±0.546
II, n = 70 (one FSH injection + PVA)	3 - 5	----	----	5/45.5	0.83±0.211	2/18.2	0.33±0.245	4/36.4	0.67±0.245	----	----
	6-10	6/6.9	0.26±0.109	15/17.2	0.65±0.162	32/36.8	1.39±0.257	24/27.6	1.04±0.235	10/11.5	0,44±0,211
	11-20	23/9.2	0.72±0.187	55/22.0	1.72±0.235	72/28.8	2.25±0.302	73/29.2	2.28±0.314	27/10.8	0,84±0,973
	>20	12/7.9	1.33±0.421	31/20.4	3.44±0.78	46/30.3	5.11±0.738	43/28.3	4.78±0.778	20/13.2	2,22±0,421
Average for group II		41/8,2	0.58±0.284	106/21.2	1.66±0.333	152/30.4	2.27±0.486	144/28.8	2.19±0.464	57/11.4	0.88±0.401
III, n =83 (one FSH injection + PEG)	3 - 5	----	----	7/38.9	0.88±0.401	11/61.1	1.34±0.558	----	----	----	----
	6-10	----	----	33/27.3	1.50±0.354	44/36.4	2.00±0.354	40/33.1	1.82±0.400	4/3.3	0,18±0,188
	11-20	17/4.4	0.43±0.208	101/26.0	2.53±0.289	133/34.3	3.33±0.389	126/32.5	3.15±0.397	11/2.8	0,29±0,205
	>20	7/3.1	0.54±0.294	59/26.3	4.54±0.930	77/34.4	5.92±0.790	70/31.3	5.38±0.986	11/4.9	0,85±0,389
Average for group III		24/3,2	0.24±0.111	200/26.6	2.36±0.333	265/35.3	3.15±0.468	236/31.4	2.59±0.289	26/3.5	0.33±0.166

As we can see from Table 4, in maximal 3-5 ovulations in group I there were no embryos at fully expanded blastocyst stage, in group II there were no embryos at early morula and fully expanded blastocyst stages, in group III there were no embryos at early morula stages, expanded blastocyst and fully expanded blastocyst stages. In addition, in group III, in maximal 6-10 ovulations there were no embryos at early morula stage. This suggests that in group III there is the greatest synchronization of the development of embryos in small limits of maximum ovulations (3-5, 6-10), in contrast to the other two groups. Moreover, in all three groups, in animals with maximum ovulations of 11 or more yellow bodies in ovaries, the presence of embryos at all stages of development is shown in Table 4.

Taking into account the fact that the most favorable stages of embryo development from the point of view of transplanted embryo survival in recipient body are late morula, early blastocyst and extended blastocyst; their total number and comparative assessment of these results in all three groups was carried out. The total number of embryos in these stages counted on average for group III (93.3%) exceeded the average number of such embryos in group II (80.4%) by 12.9%, and in group I (73.9) by 19.4%. This indicates a better synchronization of embryo developmental stages in group III where one FSH injection was used with PEG as a prolongator in comparison with group II where one FSH injection was used with PVA as a prolongator, and also with group I where eight FSH injections were performed.

Thus, as a result of the above analysis conducted in all three experimental groups on parameters of the total number of embryos in late morula, early blastocyst and extended blastocyst stages, it was found that in group III the most synchronous development of embryos which are at the indicated stages of development, is observed.

IV. CONCLUSION

According to the results of this study, it can be concluded that donor cows with poliovulation induced with "FSH-super" drug in combination with polyethylene glycol as a prolongator for follicle-stimulating hormone, had the highest polyovulatory response of ovaries, the number of proper embryos was increased and the number of degenerated embryos was reduced, a high level of ovum fertilization was obtained. In addition, a high number of excellent and good quality embryos was obtained which were suitable not only for transplantation to recipients in a freshly obtained form, but also – and it is important – for cryopreservation. The highest synchronicity of embryo development at such stages as late morula, early blastocyst, and expanded blastocyst which are generally recognized as the best ones for transplantation, was also noted. From the practical point of view, it is possible to use "FSH-super" drug combined with high molecular weight biologically degradable polymer – polyethylene glycol with molecular weight of 6000 Da – in order to get the maximum number of proper embryos from a donor cow per one session of hormonal stimulation.

V. Acknowledgement

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