the timing of an ovulatory stimulus (GnRH or pLH) resulted in an increased super stimulating response and embryo production following FTAI of lactating Holstein cows [24]. The percentage of follicular ovulation in treatment A is 70.37 % and in treatment B is 50 % (P<0.05). This present study is similar to Mikola and Taponen were 64.0 % and 62.4 % in falltropin and pluset , respectively. Cows that were treated with GnRH rather than EB at the initiation of a synchronization protocol tend to have better P/AI, probably due to increased circulating P4 concentrations during growth of the ovulatory follicle. The increased P4 was due to greater ovulation to the GnRH than EB at the start of the protocol and reduced premature CL regression for GnRH compared with EB.[8],[27].

B. Embryo Quality, transferable embryo percentage, and degenerated embryo of Local pesisir cows

The grade of embryo after flushing of local pesisir cows range from very good (6 embryos) , good (6 embryos) , unfertilized embryo (3 embryos) and degenerate embryo (4) in treatment A (Fig. 5), however in treatment B from 6 embryos recovery 2 grade of embryo were unfertilized (2 embryos) and degenerate embryo (4 embryos). The quality of embryo of pesisir cow is significant higher (P<0,05) in treatment A than treatment B. In this study found that all embryo in treatment B were the nontransferable embryo, caused the unsuccessful of embryo flushing. This result supported by [28] that there was no correlation between circulating concentration of estradiol at TAI and embryo or embryo stage. Embryo evaluation is one of the most critical steps of the embryo production. Grade 1 embryos survive well to freezing and/ thawing, whereas grade 2 and 3 must be transferred fresh into recipients [29]. The farm management and donor age are the main factors that should be considered when implementing a program of embryo transfer in Nelore cows submitted to superovulation treatment. Senile cows (≥14 years) produced on average 5.0±0.2 fewer total embryo and 3.0±0.1 fewer transferable embryo than young cows (P<0.01). donor age negatively effected the number and quality of embryo [9]. The quality of embryos and the developmental stage were similar between the group (falltropin vs pluset) and there was no difference in the proportion of low – responding donors in group F and group P, and also equal numbers of transferable embryos and pregnancies can be achieved with Folltropin and pluset [30].

The number of transferable embryo in treatment A was highly significantly (P<0.01) on grade 1, grade 2,and transferable embryo . The percentage of transferable embryo in treatment A is 66.11 % higher than in the treatment B is 0 %. The percentage of degenerate embryo is 22,22 % is and lower than treatment B 66.66 %. This result found that the treatment A is the best grade of embryo and than treatment B. The number of transferable embryo in this study was lower than reported by [24] is 66.1±8.9 % to 88.5±89 %. The present study found the is similar result of transferable embryo range from 37.8 % in cow and 69.5 % in heifer [31]. This result indicated that the quality of embryos affected by using combination of protocol to induce development of emergence wave of diameter size of dominant follicle in embryo production in donor cows.

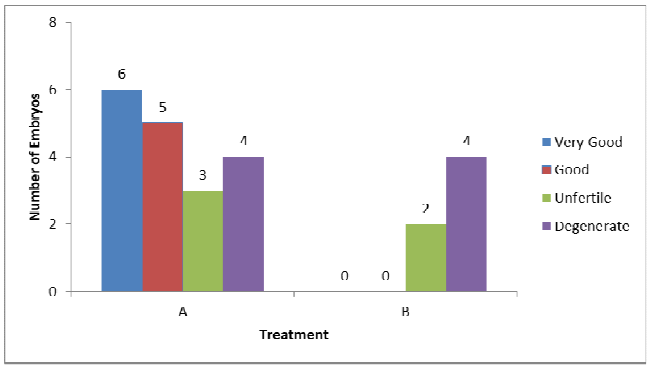

Fig. 5 The quality of embryos of local pesisir cows in GnRH plus progestetone (Treatment A) and estradiol plus progesterone (treatment B)

The degenerated embryo in treatment A is lower (22.22 %) than treatment B (66.665) , the proportion of degenerated embryos was similar between cows and heifer were 9.2 % and 11 % , respectively [31] the proportion of degenerate embryos was similar between cows and heifer were 9.2 % and 11.9 %, respectively [31] and to [30] is 65 in folltropin and 60 in pluset and the degenerated embryo range was higher is 19 % in folltropin and 25 % in pluset. In this result the quality of embryo in both treatment is lower , caused of the quality of CL i.e the size of CL is small category. The importance finding of the present study was the association between the environmental and production efficiency. In addition, that extending the period of follicular development during superovulation (from 6 to 9 days between wave emergence and ovulation) increases follicular maturation, and the number and synchrony of ovulations without compromising ova/embryo competence, and in turn, the potential for more transferable embryos [32]. Furthermore, that administration of (25mg) FSH for superovulation in donor of local Omani breed decreased number of unovulated follicles, decreased number of unfertilized ova, and increased number of transferable embryos and had beneficial effect on embryo quality compared with dose of (25 mg) and 30 mg) FSH. it could be suggested, that dose of (25mg) FSH for superovulation of the donor cows was the best and recommended for obtain higher number of good quality embryos for embryo transfer and realization of breeding programs [33]. In this present study the dose of (20mg) FSH per donor cows, depended to body weight of donors. The local pesisir cows was small category of body weight. This result was supported by (33) that un-ovulated follicles and number of unfertilized ova compared with the cows in G1(20mg), G2(25mg) and G3 (30mg) were (3.17, 4.16 and 6.83) and (3.00, 3.33 and 5.67/ flushed cow) , respectively.

The present study found that the strategies embryo production in local pesisir cows can promote superovulation donor without estrus detection (named ovsynch), synchronization of follicular wave emergence, timed of ovulation induction and AI and type, dosage and of hormone using for superovulation. The success of in vivo embryo production is closely associated to embryo quality. Therefore, factors related to breed, heat stress, and nutrition should be considered before applying SOP in the field [4].

C. Linear Regression between dominant follicle, CL and number of embryo of local pesisir cows.

The linear regression for the number of follicle dominant and the number of CL (Fig. 6), with high coefficient determination (R^2 = 0.950) and linear regression between dominant follicle with the number embryos and high coefficient determination (R^2 = 0.879). At the time of flushing not all the donor have the equal number of follicle with the CL and embryo is tend to decrease the number, even the linear regression. In treatment GnRH plus P4 from 27 follicle dominant produced 19 CL and produced 18 embryo. In treatment B at the time of flushing the number of embryos was 6 from 9 and 10 follicle dominant. This present study showed that the ovarian activity response, tend to decrease at the step of cyclic status of reproduction of donor. This means the number of embryo production depend to the number of follicular ovulation and the number of CL at the flushing. The largest number of cows showing estrus in GnRH group may be could be explained due to the induction of an earlier follicular wave. Thus, the influence of the side of previous pregnancy persisted until the second postpartum ovulation, and this affected postpartum dominant follicle selection and ovulation, but not the development of growing follicles [34]. In addition, postpartum ovarian activity imbalance was not association with the reproductive and productive performance of cows [35]. The relationship between the dominant follicle and CL due to the hormonal concentration such as estrogene and progesterone. Follicular diameter on the day of GnRH application similar to prematurely regressed CLs of the same group, but had the smaller area, this result in smaller number of luteal cells, a fact that was possible responsible for lower concentration of P4 produced by these CLs (36). This study showed the high coefficient of correlation between the number of follicular ovulation and the number of CL/ the number of embryos. Otherwise, a low correlation was observed between follicular diameter and CL category (R^2= 0.15), CL category and P4 concentration (R^2=0.19) [36]. The number of embryos collected per CL, however was significantly lower in the early versus late group (0.39±0.32 % vs 0.44±0.34%,respectively). The late collection allowed the retrieval of full concept uses (embryonic and extra embryonic tissues), even at very late stages such as days 18 to 21. [37].

This present study the number of embryos is lower than reported by[38] was 14,3 ±2.1 90.5 % (in hyaluronan 0.5%) and 14.4±2.0 (in hyaluronan 10%) and 10.2±1.8 (in FSH).The largest number of cows showing estrus in GnRH group may be could be explained due to the induction of an earlier follicular wave. Thus, expression of estrus during protocols for TAI or TET is associated with an increase in fertility and reduction in pregnancy loss. During TAI programs, optimizing follicle diameter and increasing circulating P4 on d 7 after AI were also associated with increased fertility, independent of expression of estrus [39]. This present study supported by [40] that the number of ovulatory follicles increased, the number of CL, also increased (r= 0.84) and the number of CL increased ,also increased the number of embryo (r= 0.38). thus more total embryos collection because more total structure ovarian were recovered. According to [41] that a positive correlation was found between the number of follicle responsive to pFSH (2-8mm) at the beginning of treatments and the super ovulatory response, and no differences were found in these follicular population between two treatment groups. . In addition, that no effect of the number ovulation on the percentage of transferable embryos. In this result showed that the greater number of follicle , tend to greater number of CL and embryos production and treatment GnRH plus P4 more embryo production than treatment estradiol plus P4. In addition by [42] that large ovulatory follicles have associated with the formation of the larger CL with greater capacity of synthesizing P4. The number of follicles of diameter 5 mm or less was negatively correlated with the number of follicles of diameter 6 mm or greater through the treatment period (r=-0.70,P<0.001 in the control group and r= -0.76, P<0.001). [43]. The number of large follicles at ovulations and CL occurred with lengthened protocol (7-day) than with the convention 4-day FSH treatment [32]. When animal are bred following a TAI protocol, where ovulation is induced by GnRH estrus response and increased pre-ovulatory concentration of estradiol before ovulation are critical determinants of subsequent embryo quality and potential pregnancy loss. According to [44] that inbred animals with a lower coefficient Fx had a very small and insignificant difference in quality and proportion of transferable embryos compared with group Fx=0. At greater inbreeding coefficients (3.1%- 25%),%), the reduction in embryo quality increased. Breeding management in local cow is needed to increase the reproduction efficiency by introducing AI and embryo transfer programme. This study found that the number of dominant follicle , the number of CL a significant impact on the yield of embryo production in local south pesisir cows. This means when the number of CL increase , the number of embryo significant will be increase. This present study supported by [46] that as a follicle size and concentrations of progesterone and estradiol in the follicular fluid showed significant correlations with the later development of a corpus luteum as well as morphology and progesterone concentration.

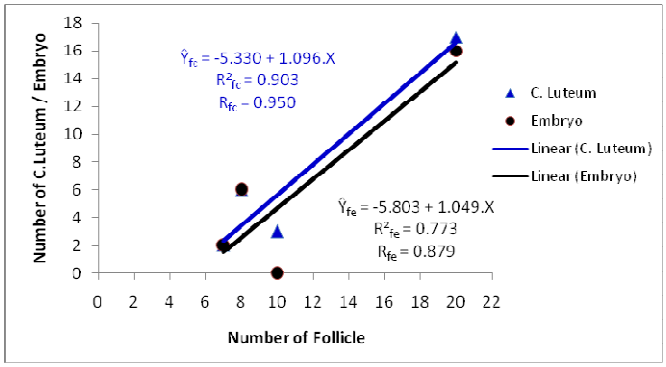

Fig. 6. Regression linear of dominant follicle, CL and embryo of local pesisir cows.

The donor of south pesisir cows had the high inbreeding without breeding management control, caused of limited bull to bred the cow at the time breeding seasons. This caused the low quality of embryos and untransferable embryo production. This result supported by [45] that a decrease in the count of transferable embryos in the inbreeding group

Fx= 3 %- 5.9%. The effect of inbreeding depression on ovarian activity in super ovulated cows. The regression equation was significant for transferable embryos was`R^2= 0.91 [46]. In this result indicated that uncontrolled of breeding programme in rural farm increased inbreeding depression caused the number of embryos production and the number of transferable embryos had a negative impact on reproduction. Based on the discussion above, we conclude that in embryo production program in local pesisir cows submitted to a superovulation protocol, special attention should be given to management and age of donors cows.In addition, when only a few CL are present in the ovaries, transrectal palpation is sufficient to accurately quantify the number;[30]. This result is indicated that the ovarian response is high relationship in fertility such as the embryo production and quality of local south pesisir cows.

IV. CONCLUSION

The strategies to increase the fertility of local Pesisir cows by using the GnRH plus progesterone combination (treatment A) in the embryo production program. This result indicated that the use of GnRH increase the embryo production and quality of embryo in local pesisir cow. Linear regression between the number of the dominant follicle, number of CL and the number of embryo with high determination coefficient (R^2) range from 0.8 to 0.9.

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REFERENCES

[1] Zaituni, U, Hendri, M. Masrizal. 2017. Fertility in south pesisir cows following ovsynch and cosynch protocols of estrus synchronization in west Sumatra. IJASEIT. 7: 2100- 2107.

[2] Rodrigues, C.A., R.M.Ferreira, L.M.Vieira, A.L. Ranier, R.L.P. Silva and P.S. Baruselli. 2011. How FTAI and FTET impact reproductive efficiency of Brazilian dairy herds. Acta Scientiae Vet. 39: 3-13

[3] Genzebu, D., 2015. A Reviewe of embryo transfer technology in cow. Global journal of Animal Science research. 3: 562-575

[4] Baruselli, P.S., L.M. Vieira, E.D.S. Batista, R.M. Ferreira, J.N.S. Sales, L.U. Gimenes, J.R.S. Torres-Junior, C.M. Martins, M.F, Sa Filho, G.A. Bo. 2015. Update on embryo production strategies. Anim. Reprod. ,12: 375-382

[5] Baruselli, P.S., R.M.Ferreira, J.N.S. Sales, L.U. Gimenes, M.F. Sa Filho, C.M. Martin, C.A.Rodrigues, G.A. Bo. 2011. Timed embryo transfer programs for management of donor and recipient cow. Theriogenology 76:1583-1593

[6] Bo,G.A., P.S.Baruselli, P.Chesta, C.M. Martin. 2006. The timing of ovulation and insemination schedules in superstimulated cow. Theriogenology. 65:77-88

[7] Lopez,H. 2005. Reproductive hormones and follicular growth during development of one or multiple dominant follicle in cow. Biol. Reprod. 72:788-795

[8] Bariselli, ,P.S., M.F. Sa Filho, C.M. Martin, L.F.Naser, M.F.G. Nogueira, C.M. Barros, 2006. Superovulation and embryo transfer in Bos indicus cow. Theriogenology. 65: 77-88

[9] Silva, J.C.C., R.H. Alvarez, C.A. Zanenga, G.T, Pereira, 2009. Factors affecting embryo production in superovulated Nelore cow. Anim. Reprod. 6: 440-445.

[10] Wiltbank,M.C., and J.R. Pursley. 2014. The cow as an induction ovulation ; Time AI after synchronization of ovulation. Theriogenology 81: 170-185

[11] Mielke, L., M. da C.C.Rodrigues, M.E.Lima, D. A. V. Acosta, F.A.B. D. Pino, M.N.Correa, J.Halfen, E.G.Xavier, C.C. Brauner. 2016. Effect of GnRH or estradiol benzoate on traits during a heatsynch protocol in dairy cows. Acta Scientiae Veterinariae. 44: 1409-1415

[12] Ongubo, M.N., H.A. Rachuonyo, R.N. Lusweti, D.K.Kios, J.K. Kitilit, K.Musee, W.K.Tonui, L.K.Lokwaleput, G.O.Oliech. 2015. Factors affecting conception rate in cow following embryo transfer. Uganda Journal Agricultura Sciences, 16:19-27

[13] Ferraz, P.A., C.Burnley, J.Karanja, A.Viera-Neto, J.E.P.Santos, R.C.Chebel, K.N.Galvao, 2016. Factors affecting the success of a large embryo transfer program in Holstein cow in a commercial herd in the southeast region of the United State. . theriogenology. 86: 1834-1841

[14] Bo, G.A., B.E.Bishop, J.M. Thomas, M.R.Ellersieck, S.E. Poock, M.E.F. Smith, D. J. Patterson. 2017. Comparing strategies to synchronize estrus before fixzed-time artificial insemination in primiparous 2-year-old beef cows. Theriogenology 87: 306-315.

[15] Pfeifer, L.F.M., W.B. Roddrigues, K.C.da Silva, N.A.Anache, N.A. Castro, E.M. Castilho, E. Nogueira, 2018. Different protocol using PGF2αas ovulation inducer in Nelore cows subjected to estradiol-progesterone timed AI based protocols. Theriogenology 120: 56-60.

[16] Ararooti,T, A.Niasari-Naslaji, B.A. Moghddam, K.Razavi, F. Panahi. 2018. Superovulation response following FSH,eCG-FSH,and hMG andpregnancy rates following transfer of hatched blastocyst embryos with different diameter and shape in dromedary camel. Theriogenology. 106: 149-156

[17] Dahlen, C.R., A.DiCostanzo, A.R. Spell, and G.C.Lamb. 2012. Use of embryo transfer seven days after artificial insemination or transferring identical demi-embryos to increase twinning in beef cow. J.Anim. Sci. 90:4823-4832.

[18] Baruselli,P.S., RM.Ferreira, M.F.Sa Filho, L.F.T. Naser, C.A. Rodrigues, C.A.Bo. 2010. Bovine embryo transfer recipient synchronization and management in tropical environments. Reprod. Fertil. Develop. 22: 67-88.

[19] Kawate, N., M.Sakase, K. Watanabe, M. Fukushima, M.Noda, K. Takeda, S. Ueno, T. Inaba, K. Kida, H. Tamada, and T. Sawada. 2007. Ovsynch plus CIDR protocol for timed embryo transfer in suckled postpartum Japanese black beef cows. J. Reprod. Dev. 53: 811-817

[20] Chankitisakul,V., J.Pitchayapipatkul, P. Chuawongboon, D. Rakwongrit, D. Sakhong, W. Boonkum, T. Vongpralub. 2017. Comparison of three superovulation protocols with or without GnRH treatment at the time of artificial insemination on ovarian respose and embryo quality in Thai native heifers. Tropical Animal Health and Production , 49: 633-659

[21] Bo, G.A., J.J.de la Mata, P. S. Baruselli, A. Menchaca. 2016. Aternative programs for synchronizing and resynchronizing ovulation in beef cow. Theriogenology; 86:388-396

[22] Carvalho, P.D., K.S.Hackbart, R.W. Bender, G.M. Baez, A.R. Dresch, J.N. Guenther, A. H. Souza, P.M. Fricke. 2014. Use of a single injection of long- acting recombinant bovine FSH to superovulate Holstein heifer: A preliminary study. Theriogenology. 82: 481-489

[23] Hirazumi, S, H. Nishinomiya, T. Oikawa, N. Sakagami, F. Sano, O. Nishino, T. Kurahara, N. Nishimoto, O. Ishiyama, Y.Hasegawa. 2015. Superovulatory response in Japanese Black cows receiving a single subcutaneous porcine follicle – stimulating hormone treatment or six intramuscular treatment over three days. Theriogenology 83: 466-473.

[24] Martin,C.M., C.A. Rodrigues, L.M. Vieira, R.J. Mapletoft, G.A. Bo, M.F. Sa Filho, P.S. Baruselli. 2012. The effect of timing of the induction of ovulation on embryo production insuperstimulated lactating Holstein cows undergoing fixed- timed artificial insemination. Theriogenology 78: 974-980

[25] Martin, C.M., I.C.C.Santos, R. Valentin, J.N.S.Sales, P.O.Reis, G.A.Crepaldi, P.S.Baruselli, M.J. Docchio, 2008. Effect of number of FSH administration reduction at superstimulatory response and embryo production of Nelore donor. Acta Sci. Vet. 34: 636

[26] Reis, P.O., C.M.Martin, I.C.C.Santos, P.S.Baruselli, 2010. Effect of FSHp (Fallotropin-V) administration number superovulation response and embryo production in Brahman donors. Acta Sci. Vet, 38: 736 (abstaract)

[27] Melo, L.F., P.L.J. Montelro Jr, R.S.Surjus, J.N. Drum, M.C. Wiltbank, R.Sartoni. 2016. Progesterone-based fixed-time artificial insemination protocols for dairy cows: Gonadotropin- releasing hormone versus estradiol benzoate at initiation and estradiol cypionate versus estradiol benzoate at the end.J. Dairy. Sci. 99: 1-11.

[28] Larimore, E.L., O.L.Amundson, S.L. Bird, B.J.Funnell, S.G.Kruse, G.A. Bridges and G.A. Perry. 2015. Influence of estrus at fixed – timed artificial insemination on early embryonic development in beef cow. J. Anim. Sci. 93: 2806-2812

[29] Bo, G.A., R.J. Mapletoft. 2013. Evaluation and classification of bovine embryos. Anim. Reprod. 10: 344- 348.

[30] Mikkola,M., J.Taponen. 2017. Embryo yirld on dairy cow after superovulation with Falltropin or Pluset. Theriogenology. 88: 84-88

[31] Vieira, L.M., C.A. Rodrigues, M.F. Mendanha, M.F. Sa Filho, J.N.S. Sales, A.H. Souza, J.E.P. Santos, P.S. Baruselli. 2014. Donor category and seasonal climate associated with emryo production and survival in multiple ovulation and embryo transfer prograaams in Holstein cow. Theriogenology. 82: 204-212

[32] Mapletoft, R.J., A. G. Guerra, F.C.F. Dias, J.Singh, G.P. Adams, 2015. In vitro and in vivo embryo production in cow superstimulated with FSH for 7 days. Anim. Reprod., 12: 383-388

[33] Hussein, A.M., Y.O.Al-Shakaili, A.N. Al- Ismaely, H.H. Al- Alawi, 2017. Effect of different doses of FSH on superovulation, production and quality of embryo in North Omani cow breed. Indian J. Anim.Sci. 51:8-14

[34] Kusaka,K., H.Miura, M. Kikuchi, M. Sakaguchi. 2018. Relationship between the side of pregnancy and side of subsequentovarian activity during the early postpartum period in lactating dairy cows. J. Reprod. Dev. 64:7-14

[35] Vrisman,D.P., N.M. Bastos, G.F. Rossi, N.N.. Rodrigues, L.P. B. Borges, A.R.R.Taira, C.C. Paro de Paz, G. de Paulia Nogueira, P.P. M. Teixeira, F.M.Montero, M.E. F. Oliveira. 2018. Corpus luteum dynamics after ovulation induction with or without previous exposure to progesterone in prepubertal Nellore heifer. Theriogenology. 106:60-69;

[36] Richard, C., I.Hue, V.Gelin, A.Neveux, E.Campion, S.A. Degrelle, Y.Heyman, P.Chavatte,-Palmer, 2015. Transrectal collected of bovine embryos up to day 21; An 8-year overview. Theriogenology. 83:1101-1109

[37] BO, G.A., D.R.Rogan, R.J.Mapletoft. 2018. Pursuit of a method for single administration of pFSH for superstimulation in cow: what we have learned. Theriogenology 112: 20-33

[38] Pereira, M.H.C., M.C. Wiltbank, J.L.M. Vasconcelos, 2016. Expression of estrus improves fertility and decreases pregnancy losses in lactating dairy cows that receive artificial insemination or embryo transfer. Journal of Dairy Sci. 99: 2237-2247

[39] Carvalho, P.D., K.S.Hackbart, R.W. Bender, G.M. Baez, A.R. Dresch, J.N. Guenther, A. H. Souza, P.M. Fricke. 2014. Use of a single injection of long- acting recombinant bovine FSH to superovulate Holstein heifer: A preliminary study. Theriogenology. 82: 481-489

[40] Biancucci, A., T. Sbaragli, A.Comin, L. Sylla, M. Monaci, T. Peric, G.Stradaioli, 2016. Reducing treatments in cow superovulation protocols by combining a pituitary extract with a 5% hyaluronan solution: Is it able to diminish activation of the hypothalamic pituitary adrenal axis compared to the traditional protocol. Theriogenology 85: 914-021

[41] Frade, M.C., C. Frade, M.B.Cordeiro, M. F.de Sa Filho, F.S. Mesquita, G.de P. Nogueira, M. Binelli, C.M.B.Membrive. 2014. Anim. Repro. Sci. 151: 85-90

[42] Guerra,A.G., A.Tribulo, J.Yapura, G.P. Adams, J.Singh. 2015. Lengthened superovulatory treatment in cow: evidence for rescue of follicles within a wave rather than continuous recruitment of new follicles. Theriogenology 84: 467-476

[43] Bezdicek, J., L.Standnik, A.Makarevich, E.Kubovicova, F. Louda, Z.Hegedosova, R.Holasek, J. Beran, M.Nejdloka. 2014. Effect of inbreeding on yield and quality of embryos recovered from superovulated Holstein cows. Turkish Journal of Veterinary and Animal Science. 38: 681-685

[44] Alvarez, R.H., M.V. Gualberto, J.B.P.Carvalho, M.Bunelli, 2005. Effect of inbreeding on ovarian responses and embryo production from superovulatedMantqueira breed cows. Theriogenology 64: 1669-1676

[45] Bezdicek, J., L.Standnik, A.Makarevich, E.Kubovicova, F. Louda, Z.Hegedosova, R.Holasek, J. Beran, M.Nejdloka. 2014. Effect of inbreeding on yield and quality of embryos recovered from superovulated Holstein cows. Turkish Journal of Veterinary and Animal Science. 38: 681-685

[46] Vernunet,A., J.M.Weitzel., T. Viergutz. 2013. Corpus luteum development and its morphology after aspiration of a preovulatory follicle is related to size and steroid content of follicle in dairy cows. Veterinary Medicina;58:221-229