

Induction of Superovulation and Invitro and In vivo Fertilization of Oocyte in 6-8 Weeks old Iraqi Goat

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Abstract

Twelve (12) female kids 6-8 weeks old were i/m injected daily with 12 mg progesterone for 18 days . Six kids were injected with 400 I.U. eCG(PMSG). hormone 48 hrs before last progesterone injection . While the other six kids were i/m injected with 100, 75 and 50 I.U of Pluset (FSH and LH hormones). respectively, at day 13,14 and 15 of progesterone treatment, those kids were showed atypical signs of estrous. The first and second surgical operation were done 48 and 120 hrs after last progesterone injections. During first operation each ovary of kid treated with eCG. contain 3-7 follicles , while 5-10 folloicles were counted on ovaries of kids treated with Pluset. Follicles on left ovaries were aspired for invitro maturation. Fresh active 800 million sperm were injected in uterus of each kids for in vivo maturation. Three Oocyte out of 13 aspired follicle were matured for e C G kids and 4 out of 15 follicles were matured for Pluset group. During 2nd operation the ovaries of pluset kids were found to look like net of bee and honey comb and not contained any corpus luteum. Superovulation Oocyte collections and ivtro and in vivo maturation could preformed ,but it is butter to used 3-4 months young female kids .

استحداث إفراط الإباضة والإخصاب الخارجي والداخلي لبويضات جداء المعز العراقي

بعمر 6 - 8 أسابيع

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الخلاصة

اثنا عشر من صغار إناث المعز العراقي بعمر 6 - 8 أسابيع حقنت بالعضل يوميا 12 ملغم من هرمون البروجستيرون قسمت الحيوانات إلى مجموعتين متساوية الأولى حقنت بالعضل 400 وحدة دولية من هرمون مصل الفرس الحامل 48 ساعة قبل آخر جرعة من هرمون البروجستيرون . اما المجموعة الثانية فحقنت بالعضل 100، 75، 50 وحدة دولية من مركب البلوسيت (الهرمون اللوتيني وهرمون محفز الجريبات) أيام 13، 14، 15 من أيام حقن هرمون البروجستيرون. صغار المعز المعاملة بمركب بلوسيت أظهرت علامات شبق المثالية. أجريت العملية الجراحية الأولى بعد 48 ساعة من آخر جرعة من هرمون البروجستيرون وكان كل مبيض من المجموعة الأولى يحتوي على 3-7 جريبات والمجموعة الثانية على 5 - 10 جريبات. تم سحب الجريبات الموجودة على المبيض الايسر لجميع الإناث لغرض الإخصاب الخارجي وتم حقن 800 مليون نطفة في رحم كل معزة بعد إجراء عملية

تكييف النطف و1500 وحدة دولية من الهرمون اللوتيني بالعضلة لكل معزة لغرض الإخصاب الداخلي. أجريت العملية الجراحية الثانية بعد 120 ساعة من آخر جرعة لهرمون البروجستيرون وتم سحب الجريبات الموجودة على المبيض الأيمن والأيسر للمجموعة الأولى أما المجموعة الثانية فكانت الجريبات على سطح المبيض تشبه خلية النحل. فقط 3 و4 بويضات من 13 و14 بويضة سحبت من المجموعة الأولى والثانية على التوالي تم إنضاجها خارجيا ولم يتم سحب أي بويضة ناضجة داخليا. نستنتج يمكن عمل إفراط الاباضة هرمونيا في صغار المعز والحصول على اكبر عدد من البيوض الناضجة داخليا وخارجيا ولهذا الهد ف يفضل استعمال صغار المعز من عمر 12 - 16 أسبوع .

Introduction

Mammalian ovary contains hundreds of thousands of Oocyte, the number of progeny a female produce is from donor of superior genetic merit. Recently Armstrong (2) recorded the use young calf and lamb as donors for Oocyte did not differ significantly whether matured invivo or invitro and development rate of blastocyte stages were similar to those observed for embryo derived from adults donors. The objective of present work and hormone stimulation and for Oocyte collection methods designed to produce maximum yields of viable Oocyte. From 6-8 weeks old local Iraqi females kids goat.

Materials and Methods

Twelve (12) female Iraqi goat kids 6-8 weeks old were used and synchronized by daily (12mg) progesterone injection i/m for 18 days. Before 48 hours of last progesterone injection 400 I.U. eCG (PMSG) were i/m injected to kids of group 1 while the kids of 2nd group were injected i/m with 100, 75 and 50 I.U. of Pluset (20 ml vial contain 500 I.U. FSH and 500 I.U. LH). Pluset injection beginning at day 13, 14 and 15, respectively, of progesterone treatment. Forty eight (48) hrs after the last progesterone injection using general anesthesia surgical .operation were done to all kids to expose ovaries and the follicles on the both ovaries of different size were counted, only the follicles of left ovaries were aspired using 18 G needle and 1ml syringe contain 0.1 ml MTC -199 media (Modified Tissue Culture). The Oocyte were incubated in 5% CO₂ incubator at 37c for 30-45 minutes with MEM (Minimum Essential Medium). And examined according Brain and Kay (4) for invitro fertilization (IVF). Before surgical incisions were close 800 million active sperms were injected into uterine Horne of each kid and 1500 I.U. HCG i/m was injected to each kid of both groups for invivo fertilization, all incision were closed . After 120 hrs from last progesterone injection the corpus luteum were counted on both ovaries the large follicles were aspired for Oocyte maturation . Each uterine horns were flushing three times with 10 mls of special embryo flushing media (molle haven 24040, Denmark) . The flushing media were examined for presence of Oocyte or/and embryo.

Results

four out of six kids treated with 225 I.U. [FSH and LH hormones (Pluset)] were showed a typical signs of estrous like stand to be mounted, vaginal secretion and homosexuality. After 48hrs each ovary of kids treated with eCG was contain 3-7 follicles of different size 2-5 mm. while the ovary of kids treated with FSH and LH was found to be contained 5-10 follicles of 2-6 mm. Table(1). Showed the number of follicles aspired from left ovaries only for 12 kids and number of mature Oocyte.

Table (1) Shows number of kid Oocyte aspired for invitro fertilization using MTC-100 and MEM matured media

Treat hormones	No. of follicles left ovary	No of aspired follicles	No. of incubated Oocyte	Normal Oocyte %	Abnormal Oocyte	Mature Oocyte MEM
PMSG	30	20	13	7	6	3
FSH + LH	40	25	15	10	6	4
Total	70	45	28	17	12	7

- N= 6 kids of each group.

The mean sizes of ovary in 400I.U. eCG treated kids after 48 and 120 hrs. of treatment was 1-2 cm length, 0.5-1 cm width and 0.4- 0.8 cm thickness While the mean size of pluset treated kids were found to be increased from 1-2 to-3 – 6 cm for Length, 0.5 – 1 to 3 – 5 cm for width and 0.4- 0.8 cm to 2-2.5cm for thickness for both ovaries. The mean length of follicles of first group was found to be 0.4- 0.8 cm with 0.25-0.5 ml fluids. While the mean size length of follicles in 2nd group was found to be 1-2.5 mm contained fluids 0.4- 0.7 ml and each ovary look like net of bee and honey comb, ovaries surface of these kids were occupied with follicles without any C.L. While the right and left ovaries of eCG treated group was found to be 31 follicles, on both ovaries ,respectively, and 13 CL, number of incubated Oocyte 28 while matured Oocyte was 7 (Table 1). No embryo can be seen in flushing media in both groups.

Discussions

Present Work is the first attempted in Iraq for estrous . Synchronization and super ovulation as well as Oocyte Collection from 6 – 8 weeks old kits The results of estrus synchronization and superovulations indicated the successful of hormonal therapy used in present work. Similar results was reported by Armstrong (2) in young calf and lamb as a donor for Oocyte. Also, almost the same results for estrous synchronization reported by AL- Ahmed (5) in adult Iraqi goat. While number of different follicles size and the number of Oocyte collected in present work was higher than those reported by AL- Ahmed as well as the number of different follicles size in both groups after 48 hrs similar to those reported in adult Iraqi goat (5) when eCG ,FSH and LH hormones was applied . Bessondio et, al., (6) and Coel et al., (7) which used non surgical collection. The low number of mature Oocyte (Table.1) from both groups could be due to effects of high doses of hormonal treatment and young age of kids which lead to collection of immature Oocyte from immature follicles. As well as the level of Co2, temperate (8). PH, time of incubation, osmolarity. Media which used had a large effect on invitro Oocyte maturation (9). Tsunodo and Sugie (10) and Pintado et al., (11) reported that the short time of follicles growth in immature goat due to high level and effects of hormone used in present work which leads to fail of oocyte maturation differed from that reported in calf and lamb which used more old animals (2). Flushing of uterus after 120 hrs with out any mature Oocyte collection could due to short period from semen injection to Oocyte collection as well as a weak sperm movement inside immature female reproductive tract and high secretion of reproductive tract leads to sweep of sperm and low sperm movement(12). The shape and size of kid ovaries treated with pluset (FSH

and LH hormones) ,also, indicated that large doses of treated hormones. Conclusions : Estrous synchronization and superovulation, surgical Oocyte collection could be made more successfully when using young female Iraqi goat with an age between 3-4 months.

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